The American Journal of Medicine

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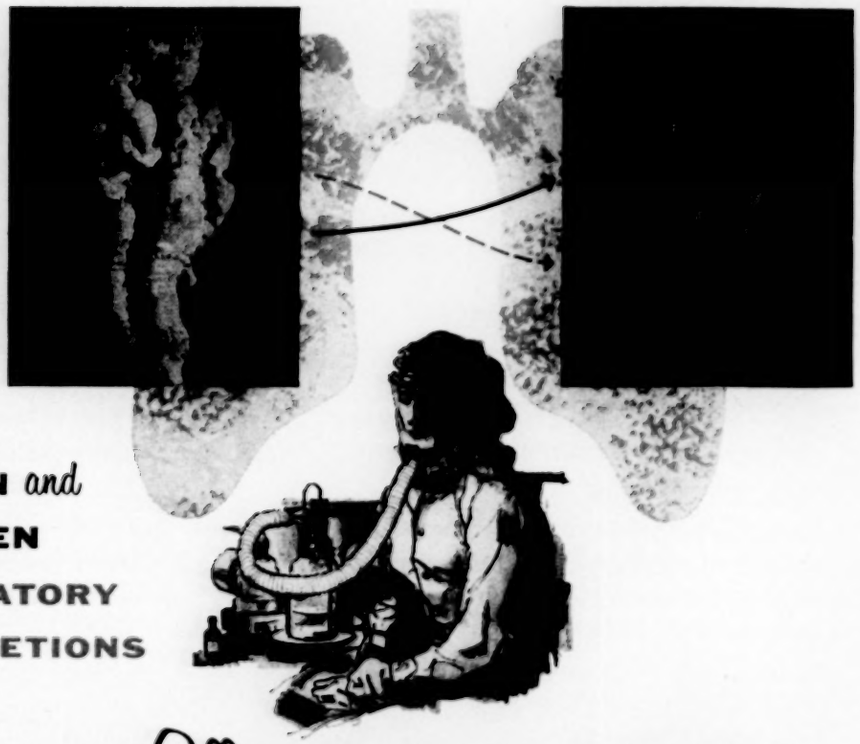

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The American Journal of Medicine

Vol. XXVI FEBRUARY, 1959 No. 2

CONTENTS

Editorial

Clinical Studies

Time has endorsed Dr. Somogyi's past contributions, and these present studies deserve equal attention, particularly since they deal with the immediate aspects of management of the difficultly controlled diabetic patient. The first paper is concerned with the elusive G/I ratio (the relation of grams of glucose utilized to units of insulin injected) as a guide to insulin requirement, and points out that this is not a fixed but a variable quantity, depending upon available glucose and many other factors. The second paper enlarges upon this theme, stressing the ineffectiveness and dangers of excessive insulin dosage aimed at abolishing glycosuria at the expense of hypoglycemia. Since "hypoglycemia begets hyperglycemia," a vicious cycle leading to higher and higher insulin dosage ensues, with exacerbation of the diabetic syndrome; the hyperglycemic rebound is attributable to release of epinephrine and other hormones which increase the blood sugar level in response to hypoglycemia. One should, therefore, adjust the insulin dosage and diet not so much to abolish glycosuria but to achieve maximal glucose utilization per unit of insulin administered. This can often be achieved, even in the "unmanageable" cases in question, by slow reduction in insulin dosage, with proper distribution in relation to the adjusted diet. Examples are cited to illustrate the striking successes in regulation that can thus be achieved, and many others in the general experience can now be adduced. The third paper carries on by discussing the phenomenon of paradoxical hyperglycemia sometimes observed in states of organic or iatrogenic hyperinsulinism. This too can be explained by accelerated release of epinephrine and other hormones capable of elevating the blood sugar.

There has been comparatively little systematic study of the precise defects in blood coagulation in patients with liver disease, and of the adequacy of replacement by transfusion with stored blood or fresh blood, despite the recent increase in surgical procedures in such patients. This investigation

Contents continued on page 5

CONTENTS continued–February 1959

VOLUME TWENTY-SIX NUMBER TWO

Contents continued on page 7

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REFERENCES: • 1. Freyberg, R. H.; Berntsen, C. A., Jr., and Hellman, L: Arth. & Rheum. 1:215 (June) 1958. • 2. Sherwood, H., and Cooke, R. A.: J. Allergy **28**:97 (March) 1957. • 3. Shelley, W. B.; Harun, J. S., and Pillsbury, D. M.: J.A.M.A. **167**:959 (June 21) 1958. • 4. Dubois, E.L.: California Med. **89**:195 (Sept.) 1958. • 5. Hartung, E.F.: J.A.M.A. **167**:973 (June 21) 1958.

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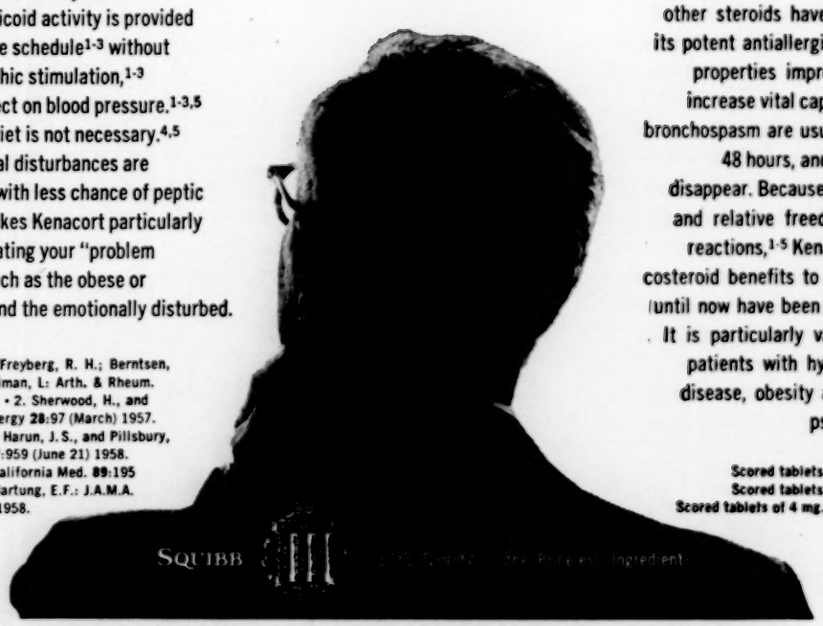

CONTENTS continued–February 1959

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Contents continued on page 9

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CONTENTS continued–February 1959

VOLUME TWENTY-SIX NUMBER TWO

Advertising Index on Page 125

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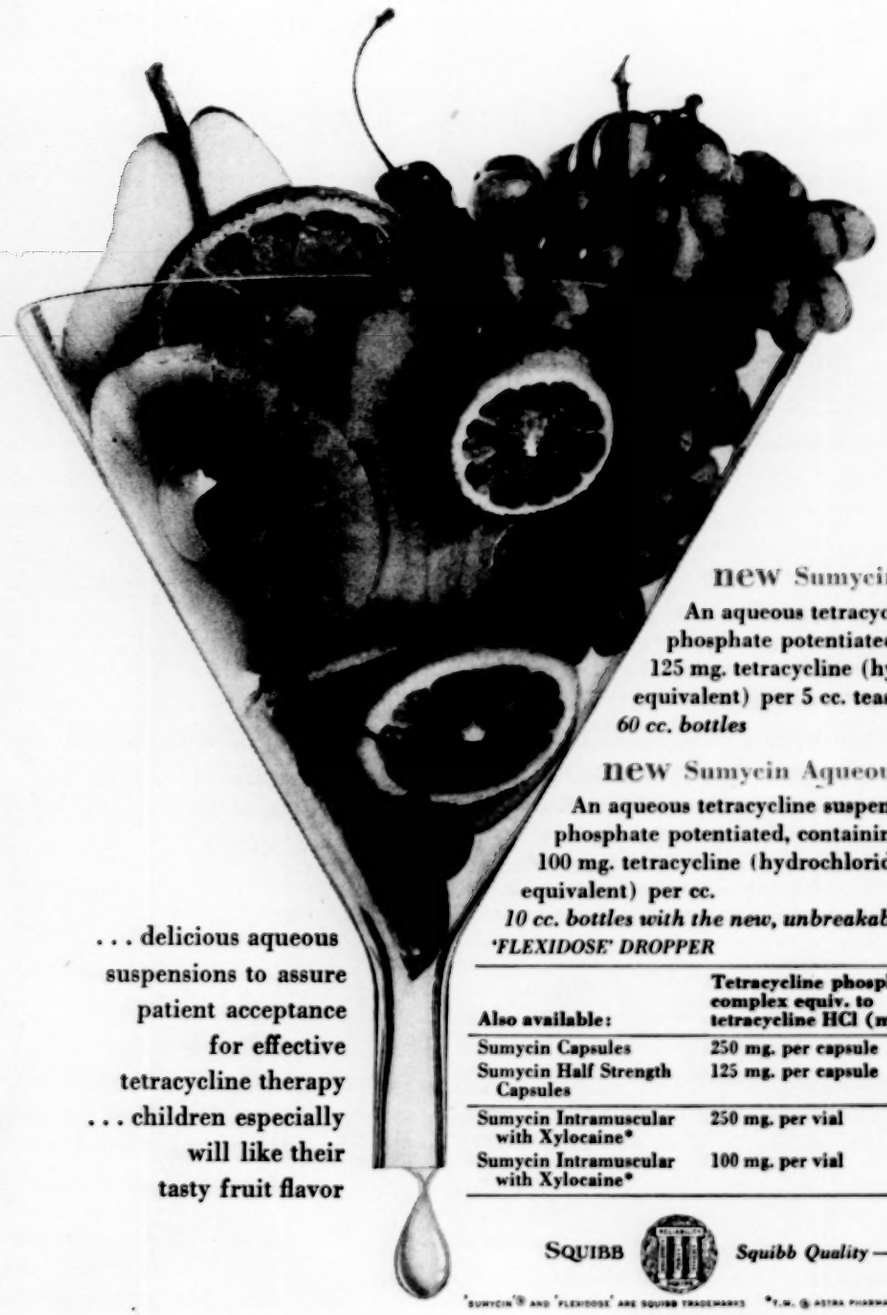

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REFERENCES: 1. Natenshon, A. L.: Dis. Nerv. System *17*:392 (Dec.) 1956. 2. Landman, M. E., Preisig, R., and Perlman, M.: J. M. Soc. New Jersey *55*:55 (Feb.) 1958. 3. Carter, C. H., and Maley, M. C.: Dis. Nerv. System *18*:146 (April) 1957.

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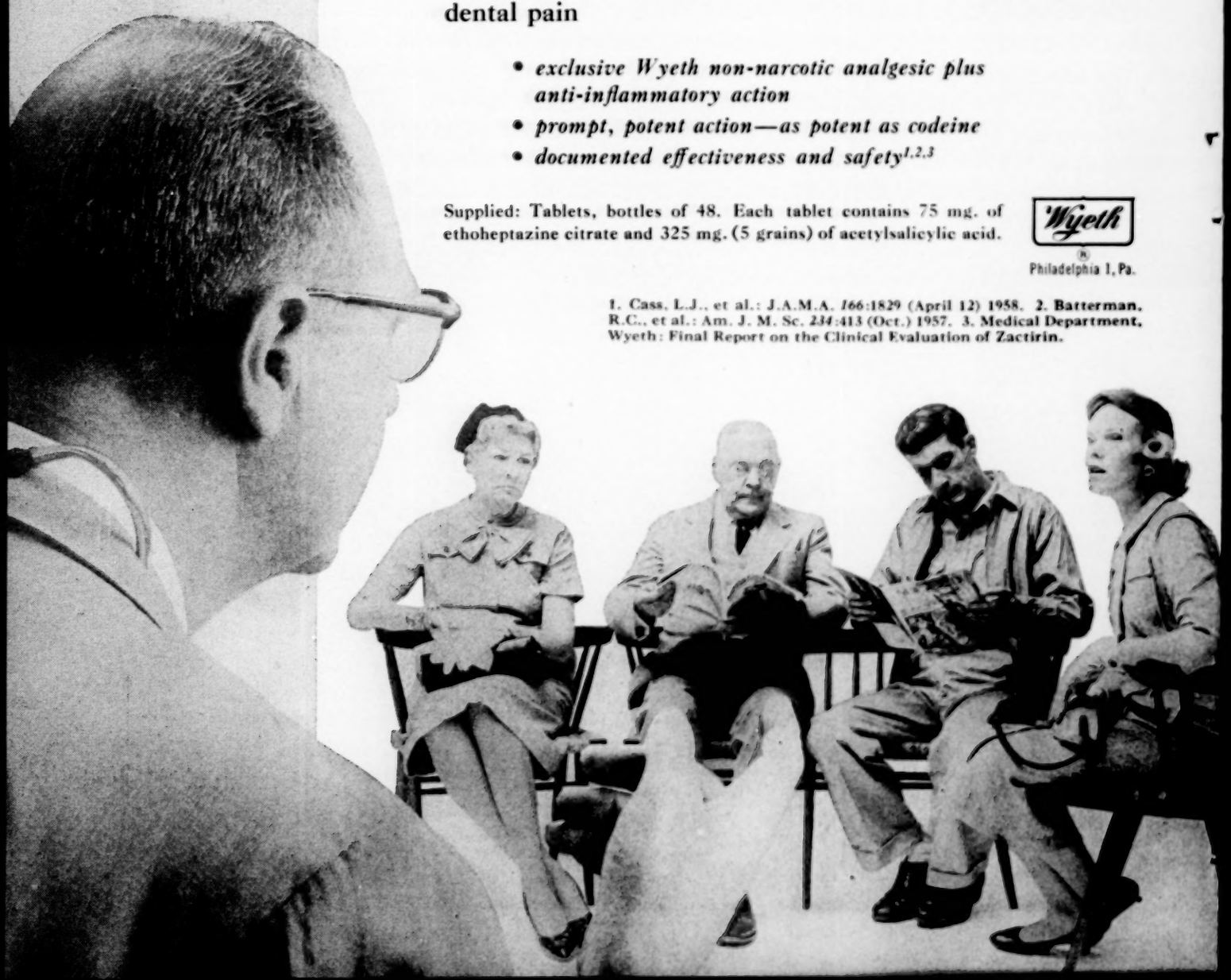
1. Cass, L.J., et al.: J.A.M.A. *166*:1829 (April 12) 1958. 2. Batterman, R.C., et al.: Am. J. M. Sc. *234*:413 (Oct.) 1957. 3. Medical Department, Wyeth: Final Report on the Clinical Evaluation of Zactirin.



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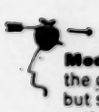

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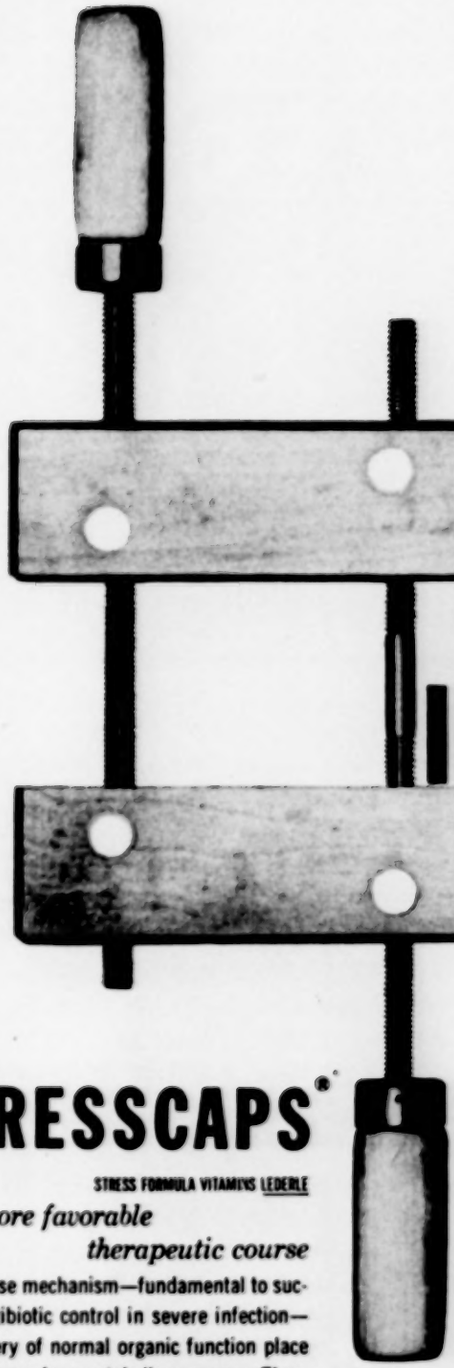

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1. Daskal, H. M.: Antibiotic Med. & Clin Ther. 2:33 (June) 1956.
2. Pollack, H. and Halpern, S. L.: Therapeutic Nutrition, National Research Council, Washington, D. C., 1952.

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1. J. D. Young, Jr., W. S. Kiser and O. C. Beyer, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **2.** H. P. Ironson and C. Patel, *Antibiotic Med. & Clin. Therapy* 6: (Suppl.), 1959 (in press). **3.** T. D. Michael, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **4.** W. A. Leff, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **5.** B. A. Koechlin, W. Kern and R. Engelberg, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **6.** R. J. Schnitzer and W. F. DeLorenzo, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **7.** R. J. Schnitzer, W. F. DeLorenzo, E. Grunberg and R. Russomanno, *Proc. Soc. Exper. Biol. & Med.* 99:421, 1958. **8.** B. H. Leming, Jr., Clyde Flanigan, Jr. and B. R. Jennings, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **9.** J. C. Elia, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **10.** W. F. DeLorenzo and R. Russomanno, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **11.** J. F. Glenn, J. R. Johnson and J. H. Semans, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **12.** B. Fust and E. Boehni, *Antibiotic Med. & Clin. Therapy* 6: (Suppl.), 1959 (in press). **13.** W. F. DeLorenzo and A. M. Schumacher, *Antibiotic Med. & Clin. Therapy*, 6: (Suppl.), 1959 (in press). **14.** S. Ross, J. R. Puig and E. A. Zaremba, *Antibiotics Annual* 1958-59 (in press). **15.** E. H. Townsend and A. Borgstedt, *Antibiotics Annual* 1958-59 (in press). **16.** W. P. Boger, *Antibiotics Annual* 1958-59 (in press). **17.** O. Brandman, C. Oyer, R. Engelberg and L. O. Randall, *J. M. Soc. New Jersey* (in press).

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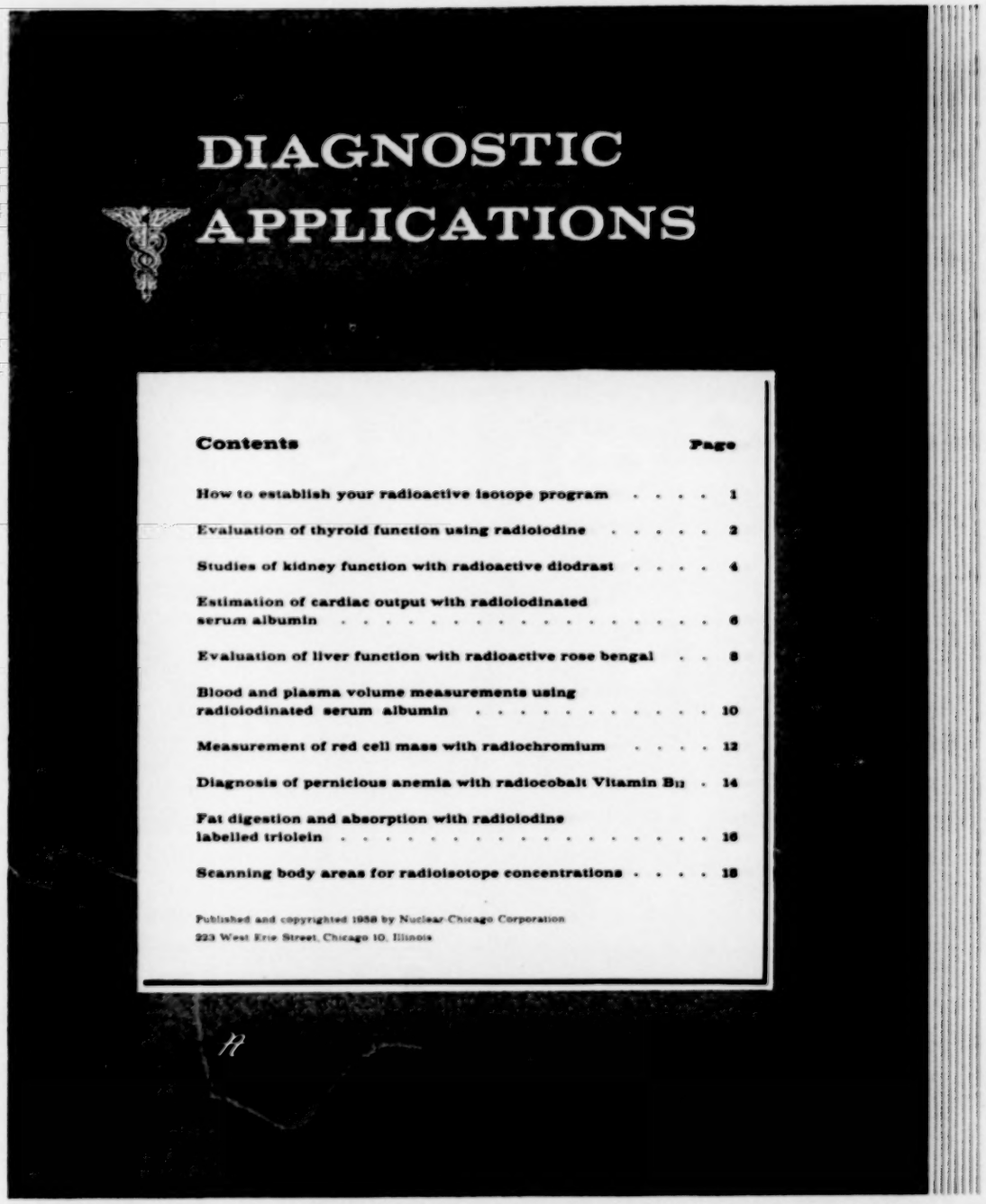

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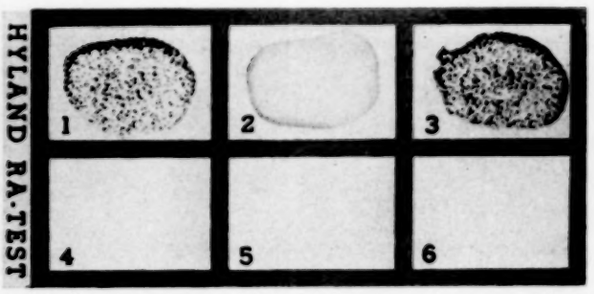

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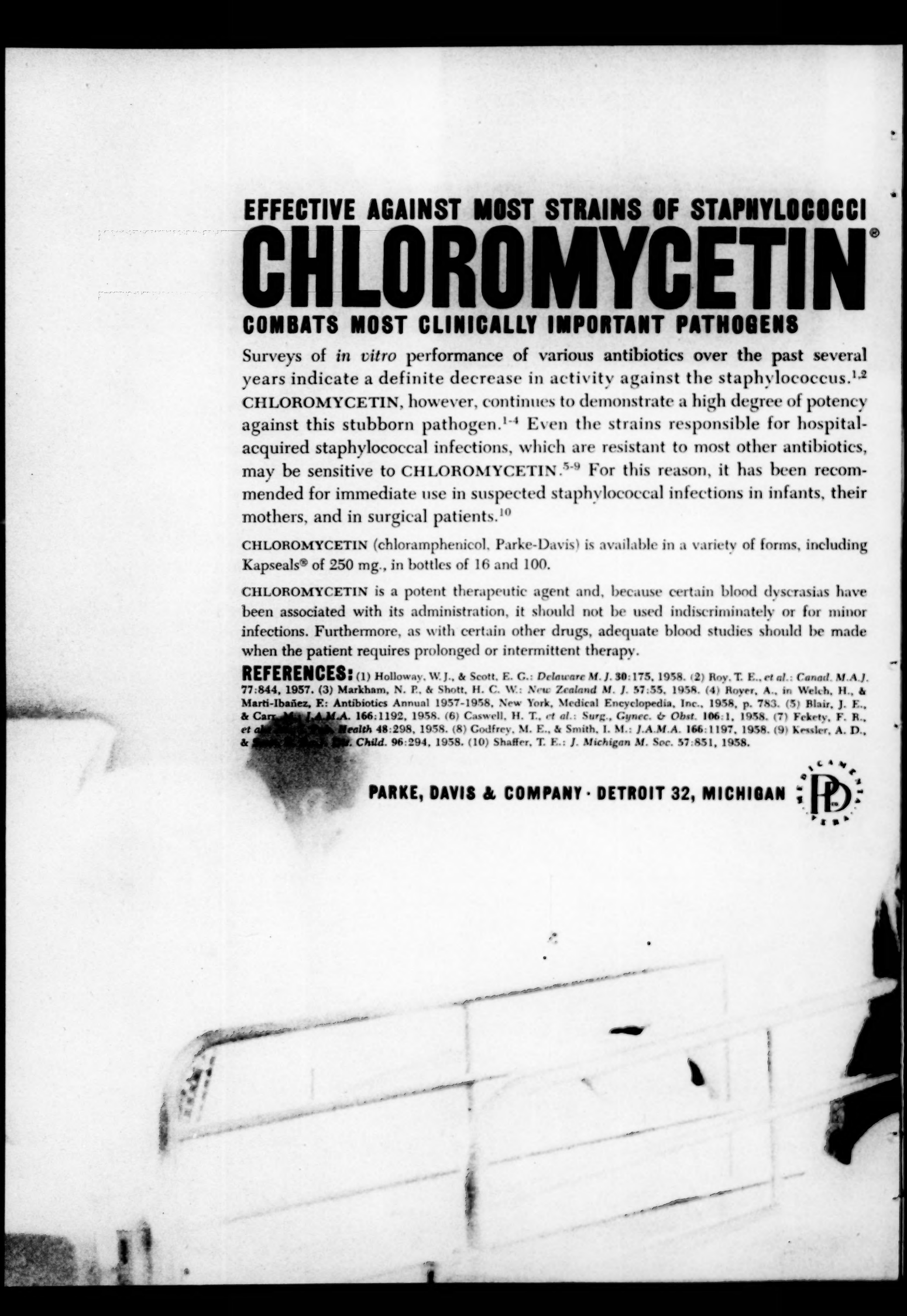

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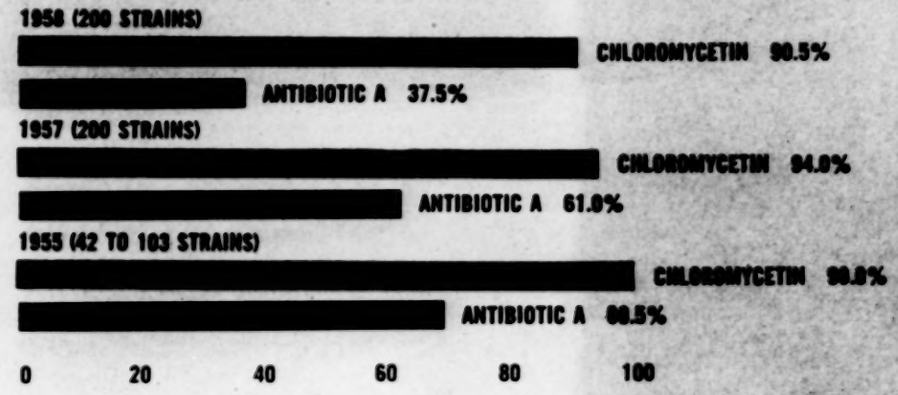

*Adapted from Holloway and Scott.[1] In this study CHLOROMYCETIN and Antibiotic A were used in identical strengths of 5 mcg.

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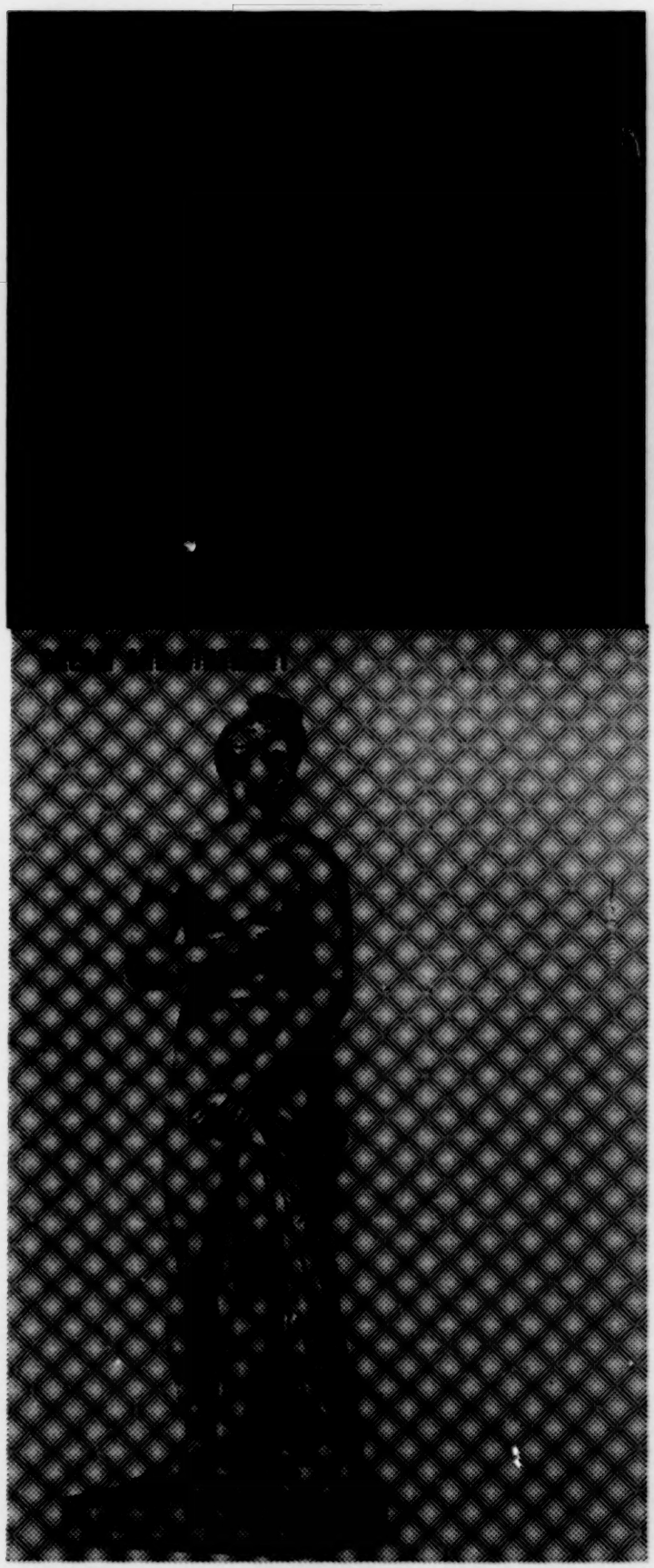

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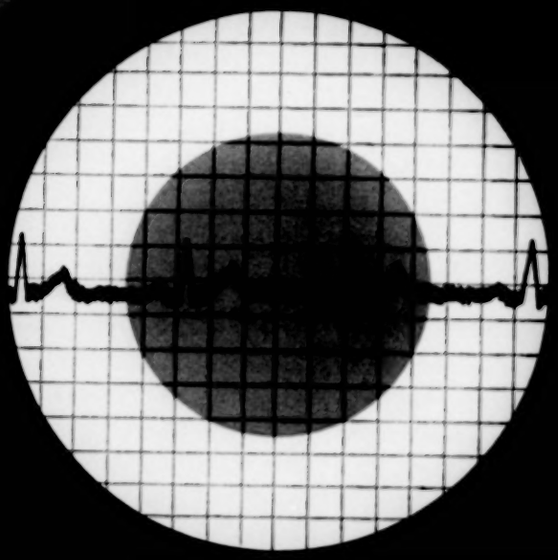

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*F. K. Garvey and J. M. Lancaster, *North Carolina M. J., 18*:78, 1957.

GANTRISIN® Brand of sulfisoxazole
ROCHE—Reg. U.S. Pat. Off.

ROCHE LABORATORIES • *Division of Hoffmann-La Roche Inc* • *Nutley 10* • *N. J.*

adsorptive power

KAOLIN

CLAYSORB is 5 times as adsorptive as kaolin

When you prescribe POLYMAGMA or POLYMAGMA Plain to control diarrhea, you are prescribing adsorptive superiority. Both preparations contain Claysorb—a new intestinal adsorbent whose superiority over kaolin has been demonstrated in exhaustive studies.[1,2,3]

For *bacterial* diarrhea, POLYMAGMA is bactericidal to many intestinal pathogens. It is soothing and protective to the irritated mucosa. It aids in the restoration of normal intestinal function. Highly effective, highly palatable.

For *nonbacterial* diarrhea, POLYMAGMA Plain—same formula but without antibiotics.

1. Barr, M., and Arnista, E.S.: J. Am. Pharm. A. (Scient. Ed.) *46*:493 (Aug.) 1957. 2. Barr, M., and Arnista, E.S.: *Ibid.* *46*:486 (Aug.) 1957. 3. Barr, M.: *Ibid.* *46*:490 (Aug.) 1957.

Polymagma®

Dihydrostreptomycin Sulfate, Polymyxin B Sulfate, and Pectin with Claysorb* (Activated Attapulgite, Wyeth) in Alumina Gel

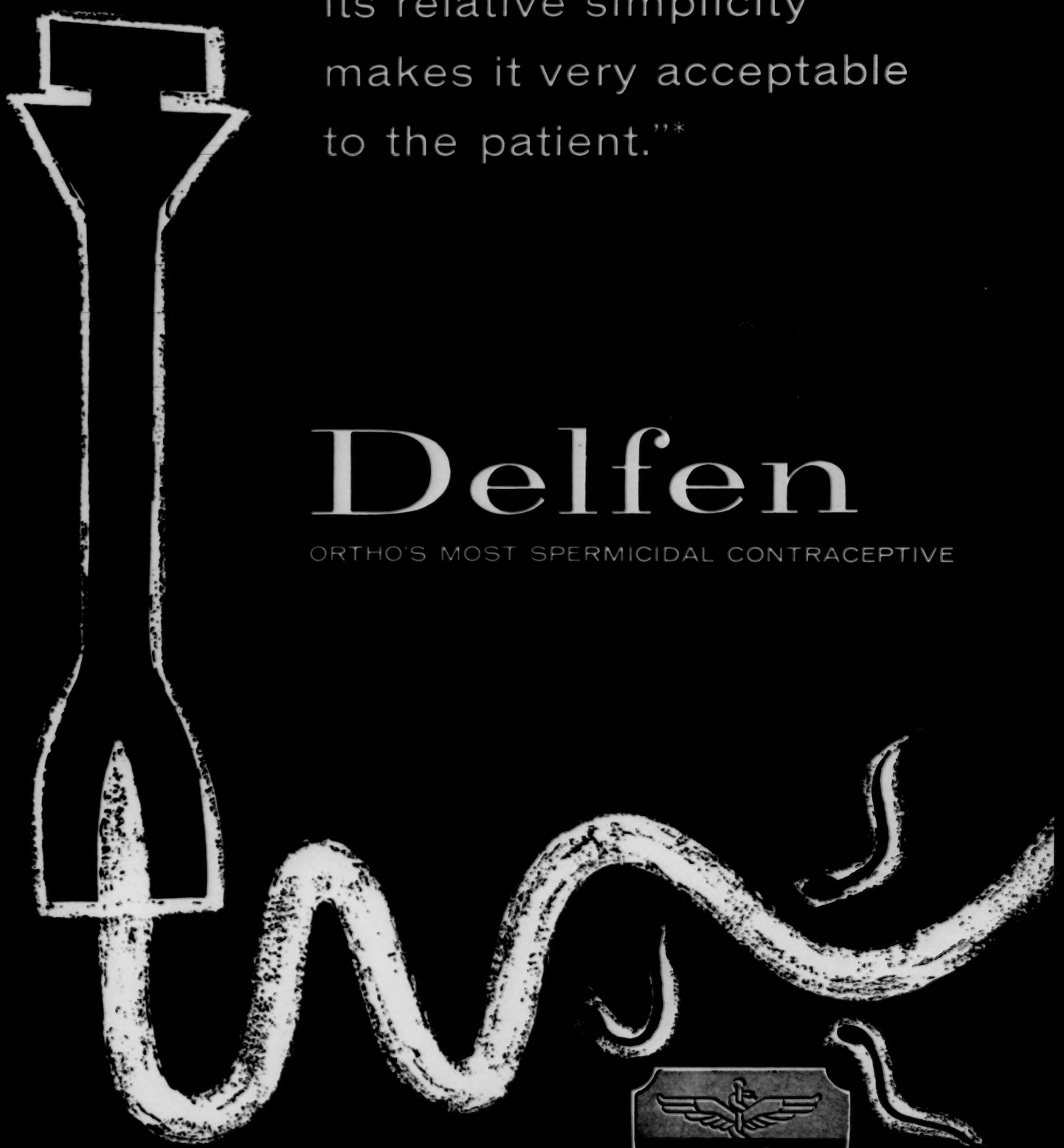
*Trademark

Wyeth®
Philadelphia 1, Pa.

PHYSICIANS' COUNCIL FOR INFORMATION ON CHILD HEALTH

This advertisement conforms to the Code for Advertising of the Physicians' Council for Information on Child Health

"Its relative simplicity
makes it very acceptable
to the patient."*



Delfen

ORTHO'S MOST SPERMICIDAL CONTRACEPTIVE

Ortho

During her reproductive years— almost every woman needs iron therapy

The average woman loses 1 mg. of iron per day through normal elimination. Add to this an average of 15 to 30 mg. each menstrual period.[1] It is evident that many women deplete their iron reserves faster than the normal diet can replenish them.

Many clinicians recommend that every woman, during her reproductive years, receive iron therapy for six weeks each year.

Livitamin, with *peptonized* iron, offers an excellent formula to restore depleted iron reserves. Peptonized iron is well absorbed and stored, and less irritating than other forms. The Livitamin formula, which contains the B complex, provides integrated therapy to normalize the blood picture.

1. Brown, E.B., Jr. *The Management of Iron Deficiency Anemia*, GP, 2:87 (Feb. 1958).

LIVITAMIN®

with Peptonized Iron

FORMULA: Each fluidounce contains:

Iron peptonized (Equiv. in elemental iron to 71 mg.)	420 mg.
Manganese citrate, soluble	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Vitamin B_{12} Activity (derived from Cobalamin conc.)	20 mcg.
Nicotinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	2 Gm.
Rice bran extract	1 Gm.
Inositol	30 mg.
Choline	60 mg.

The S. E. MASSENGILL Company

BRISTOL, TENNESSEE • NEW YORK • KANSAS CITY • SAN FRANCISCO

Livitamin is better because it contains peptonized iron

current studies show peptonized iron*

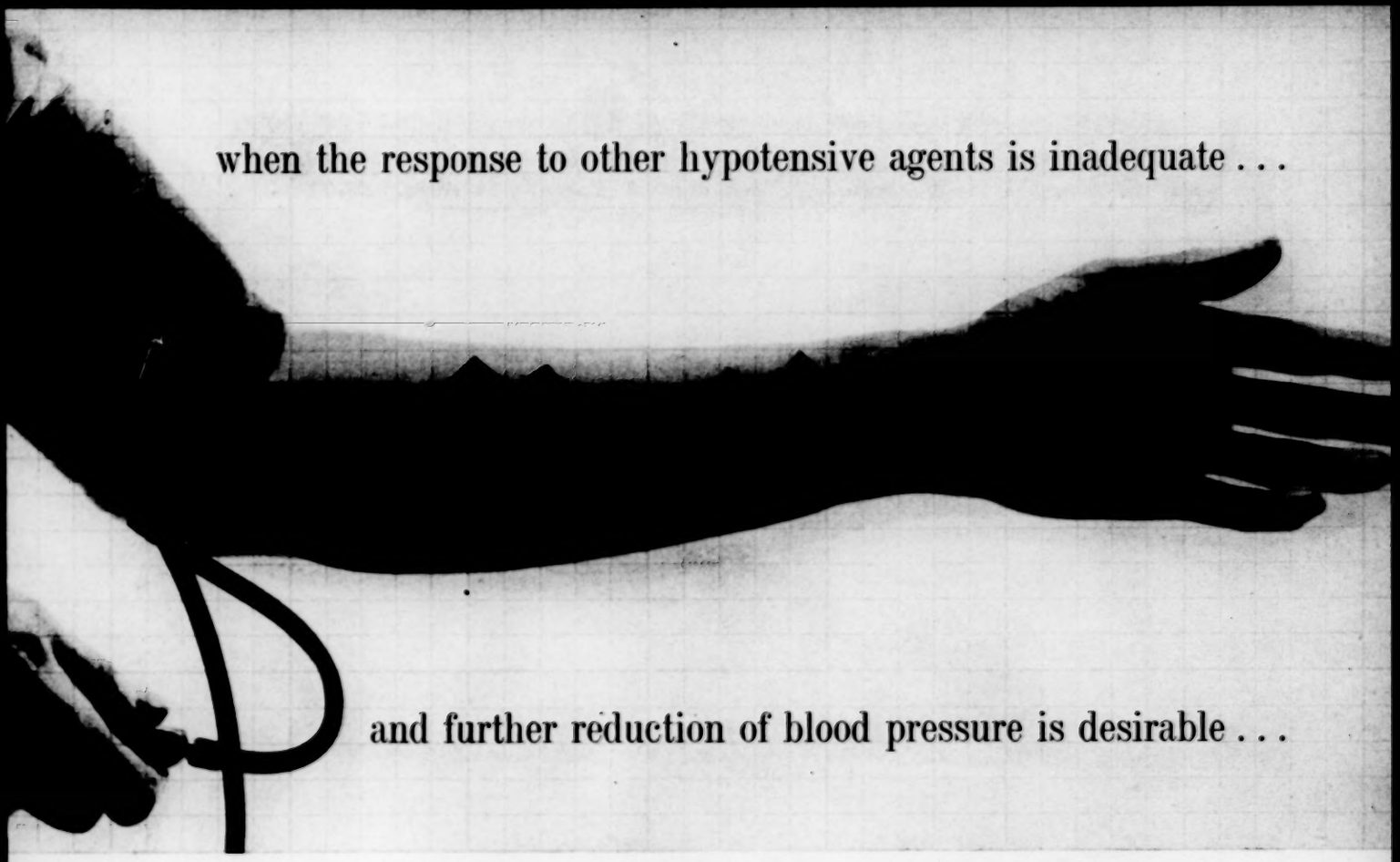
- Absorbed as well as ferrous sulfate.
- Non-astringent.
- Free from tendencies to disturb digestion. (One-tenth as irritating to the gastric mucosa as ferrous sulfate.)
- More rapid response in iron-deficient anemias.
- One-third as toxic as ferrous sulfate.

*Keith, J.H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Am. J. Clin. Nutrition *1*:35 (Jan.-Feb., 1957).

LIVITAMIN®

with Peptonized Iron

The S. E. MASSENGILL Company BRISTOL, TENNESSEE • NEW YORK • KANSAS CITY • SAN FRANCISCO



when the response to other hypotensive agents is inadequate . . .

and further reduction of blood pressure is desirable . . .

adding ganglionic-blocking

INVERSINE®

MECAMYLAMINE HCl

often makes possible a lessening of cardiovascular-renal damage, regression of the basic disease, and prolongation of life

"unnecessary delay must be avoided in establishing ganglion blockade in severe or malignant hypertension"
Beem, J. R., and Moyer, J. H.: Geriatrics 13:378, June 1958.

relieve high blood pressure and its manifestations

INVERSINE®

MECAMYLAMINE HCl

for moderate, severe, and malignant hypertension

"When employed under carefully controlled conditions with adequate attention to proper regulation of dosage, mecamylamine ['INVERSINE'] may be expected to reduce blood pressure effectively and to ameliorate various manifestations of hypertensive-cardiovascular disease. These include such symptoms as headache, dizziness, vertigo, hypertensive encephalopathy, cerebral or subarachnoid hemorrhage, retinopathy, cardiac hypertrophy and, in some cases, cardiac decompensation."

A.M.A. Council on Drugs, New and Nonofficial Drugs: Philadelphia, J. B. Lippincott Co., 1958, p. 285

A GREATLY IMPROVED GANGLIONIC BLOCKING AGENT

'INVERSINE'

- of the orally effective blocking agents, only 'INVERSINE' is completely and uniformly absorbed
- because it is uniformly absorbed, 'INVERSINE' provides predictable, reproducible effects with minimal day-to-day fluctuations
- has a gradual onset of effect, reducing the likelihood of sudden drops in blood pressure
- effective in extremely low dosage (orally, 10 mg. 'INVERSINE' is approximately equivalent to 100 mg. pentolinium, 80 mg. chlorisondamine, 1000 mg. hexamethonium)
- has a long duration of action (6 to 12 hours or longer), permitting convenient dosage schedules
- development of tolerance is not as pronounced as with other ganglionic blocking drugs
- effective in many patients who do not respond to other ganglionic blocking drugs

pretreatment with 'Diuril', or 'Diuril' and rauwolfia, enhances therapy with 'Inversine'

"Pretreatment with chlorothiazide ['DIURIL'] and rauwolfia reduces the dosage requirement, augments blood pressure response, and moderates certain of the side effects of ganglion blocking agents. Although such basal therapy is advantageous, unnecessary delay must be avoided in establishing ganglion blockade in severe or malignant hypertension."

Beem, J. R., and Moyer, J. H.: Geriatrics 13:378, June 1958

dosage recommendations for new patients

1. *Initiate therapy with 'DIURIL'*

'DIURIL' is given in a dosage range of from 250 mg. twice a day to 500 mg. three times a day, depending on severity of the hypertension.

2. *Add other agents*

Other drugs (rauwolfia, 'INVERSINE', hydralazine, etc.) are added as necessary and their dosage adjusted according to patient response.

'INVERSINE' is given in the same manner whether used with other drugs or alone. Recommended initial dosage is 2.5 mg. twice a day, preferably after meals. May be increased by 2.5 mg. at intervals of no less than two days until desired response is obtained. In severe or urgent cases, the increments may have to be larger or more frequent, with the largest dose given preferably at noon or in the evening. 'INVERSINE' is extremely potent and should always be titrated according to the patient's orthostatic blood pressure response.

3. *Adjust dosage of all medication*

The patient must be observed frequently and careful adjustment of all agents should be made to determine optimal maintenance dosage.

Precautions: Side effects of 'INVERSINE' are essentially the same as those encountered with other ganglionic blocking agents. At the first sign of constipation, vigorous treatment must be initiated immediately since paralytic ileus may result if constipation is unchecked. Patients should be informed how to cope with postural hypotension should this occur. 'INVERSINE' is contraindicated in coronary insufficiency, organic pyloric stenosis and recent myocardial infarction. Additional information on 'INVERSINE' and 'DIURIL' is available on request.

Supplied: 'INVERSINE', tablets of 2.5 and 10 mg. Bottles of 100.

'DIURIL', tablets of 250 mg. and 500 mg. Bottles of 100 and 1000.

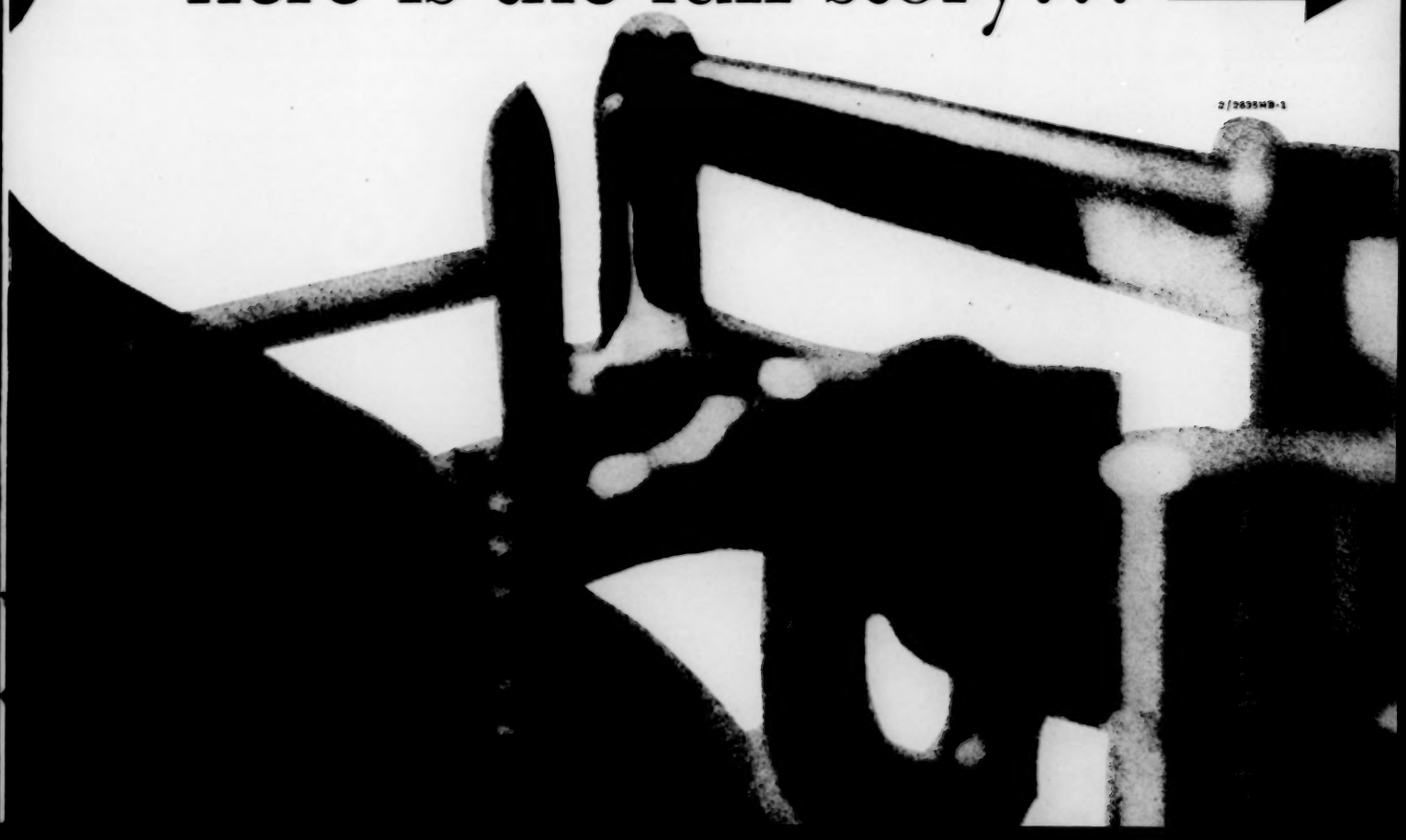
MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

The drug that lowered this patient's blood pressure *for the first time without side effects* is now available for your prescription...

Created by C I B A
World Leader in Hypertension Research

Singoserp™
(syrosingopine CIBA)

here is the full story... *please turn*



2/2835MB-1

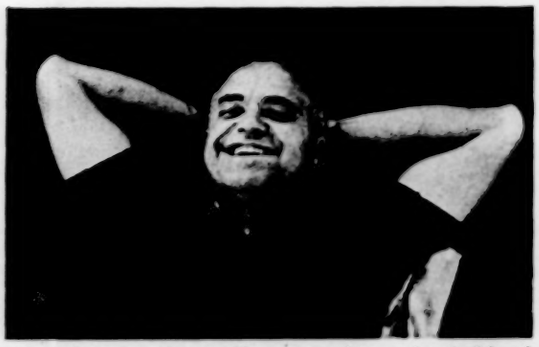
a major improvement in rauwolfia
a major advance in antihypertensive therapy

Developed after three years of basic research, proved during one of the most extensive clinical trials in pharmaceutical history, here is what* Singoserp *can do:

Patient P. K. was first seen with a blood pressure of 220/138 mm. Hg; he complained of headache, palpitation, nervous tension and hyperhidrosis.

Hospitalized briefly for observation and treatment, he was placed on a 4-Gm. sodium diet, plus chlorothiazide and mecamylamine regulated according to b.p. reading, which he was taught to take himself.

One month later his blood pressure was 140/104; he complained of dryness of mouth, chest pain, constipation and nocturia (twice a night). He was then started on Singoserp (0.5 mg. daily) with instructions to reduce the other medications to the extent possible, as evidenced by his b.p. readings.



After five months on Singoserp the patient's blood pressure ranged between 120/84 and 140/100. No mecamylamine was required; only ⅓ the original dose of chlorothiazide was required. One month later, chlorothiazide was stopped and the patient was maintained on Singoserp alone, 1 mg. b.i.d. Favorable blood pressure response continues and patient feels well. Since taking Singoserp patient reports *no* chest pain, *no* mouth dryness, *no* other side effects.

Singoserp™

(syrosingopine CIBA)

Solves the Side Effects Problem in Most Hypertensive Patients

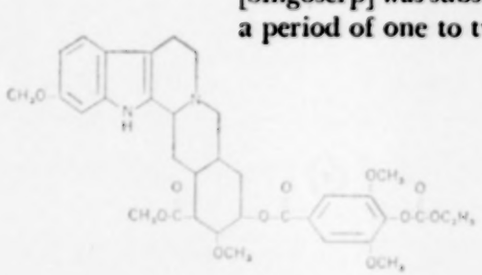
1. For new hypertensive patients Singoserp is the ideal antihypertensive drug for new patients because it lowers blood pressure without creating the side effects problem posed by conventional rauwolfia agents.

2. For hypertensive patients already undergoing drug treatment Singoserp, added to any antihypertensive regimen, makes it possible to maintain blood pressure levels achieved with more potent agents, while reducing their dosage requirements—or even eliminating them altogether in some cases.

Infrequent side effects—"The chief advantage of [Singoserp] over other Rauwolfia derivatives seems... to be the relative infrequency with which it produces disturbing side effects."[1]

Less sedation—"It [Singoserp] is approximately equipotent to reserpine as a hypotensive agent but is definitely less sedative or tranquilizing."[2]

Depression relieved—"In those patients who had been depressed, [Singoserp] was substituted for other Rauwolfia preparations and within a period of one to two weeks this depression was relieved."[3]



Created in the laboratory by altering the reserpine molecule so as to preserve its antihypertensive property and virtually eliminate its undesirable side actions.

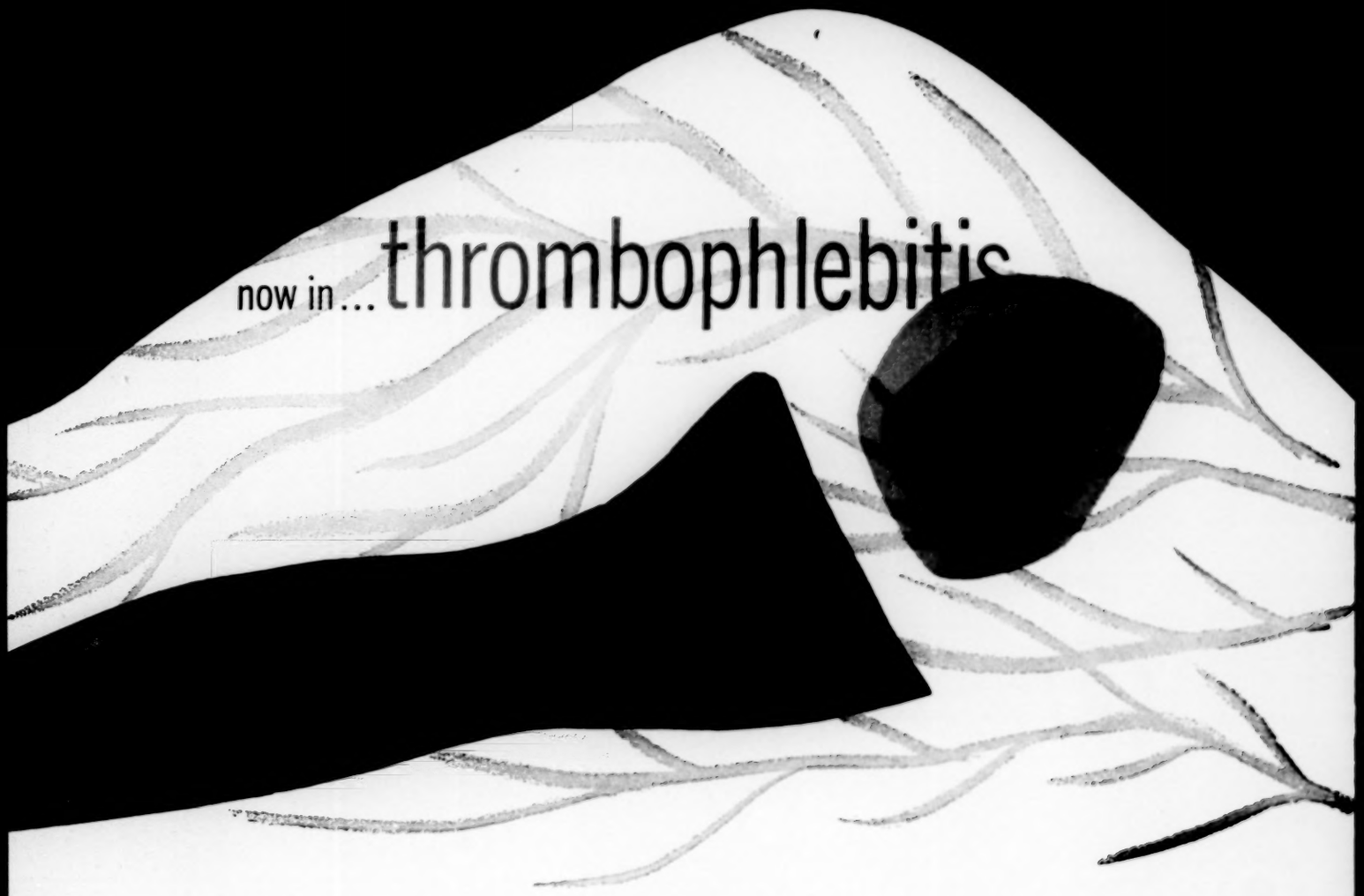
Dosage: *In New Patients:* Average initial dose, 1 to 2 tablets (1 to 2 mg.) daily. Some patients may require and will tolerate 3 or more tablets daily. Maintenance dose will range from ½ to 3 tablets (0.5 mg. to 3 mg.) daily. When necessary for adequate control of blood pressure, more potent agents may be used adjunctively with Singoserp in doses below those required when they are used alone. *In Patients Taking Other Antihypertensive Medication:* Add 1 to 2 Singoserp tablets (1 to 2 mg.) daily. Dosage of other agents should be revised downward to a level affording maximal control of blood pressure and minimal side effects.

Supplied: Singoserp *Tablets,* 1 mg. (white, scored); bottles of 100.

References: 1. Herrmann, G. R., Vogelpohl, E. B., Hejtmancik, M. R., and Wright, J. C.: To be published. 2. Wolffe, J. B.: Mod. Med. *26*:253 (Feb. 1) 1958. 3. Bartels, C. C.: To be published.

9/3656MR-2

CIBA
SUMMIT, N. J.



"early and marked regression"[1]

in acute superficial thrombophlebitis

BUTAZOLIDIN®

(phenylbutazone GEIGY)

nonhormonal anti-inflammatory agent

Relieves Pain Rapidly—BUTAZOLIDIN usually produces complete relief of pain within 24 hours or less.[1,2]

Resolves Inflammation—Fever subsides and local heat, tenderness and swelling regress quickly.[1,3,4] "In the majority of cases there was complete resolution by the fourth day."[5]

Permits Early Ambulation—"As a rule within 24 hours, most patients were able to get up and walk about...."[1] This rapid response to BUTAZOLIDIN greatly reduces disability and economic loss for patients.

Short Course of Treatment—Most patients require only from 2 to 7 days' therapy.[1,5]

BUTAZOLIDIN® (phenylbutazone GEIGY). Red coated tablets of 100 mg. BUTAZOLIDIN Alka Capsules, each containing BUTAZOLIDIN 100 mg.; aluminum hydroxide 100 mg.; magnesium trisilicate 150 mg.; homatropine methylbromide 1.25 mg.

BUTAZOLIDIN being a potent therapeutic agent, physicians unfamiliar with it are urged to send for literature before instituting therapy.

GEIGY
Ardsley, New York

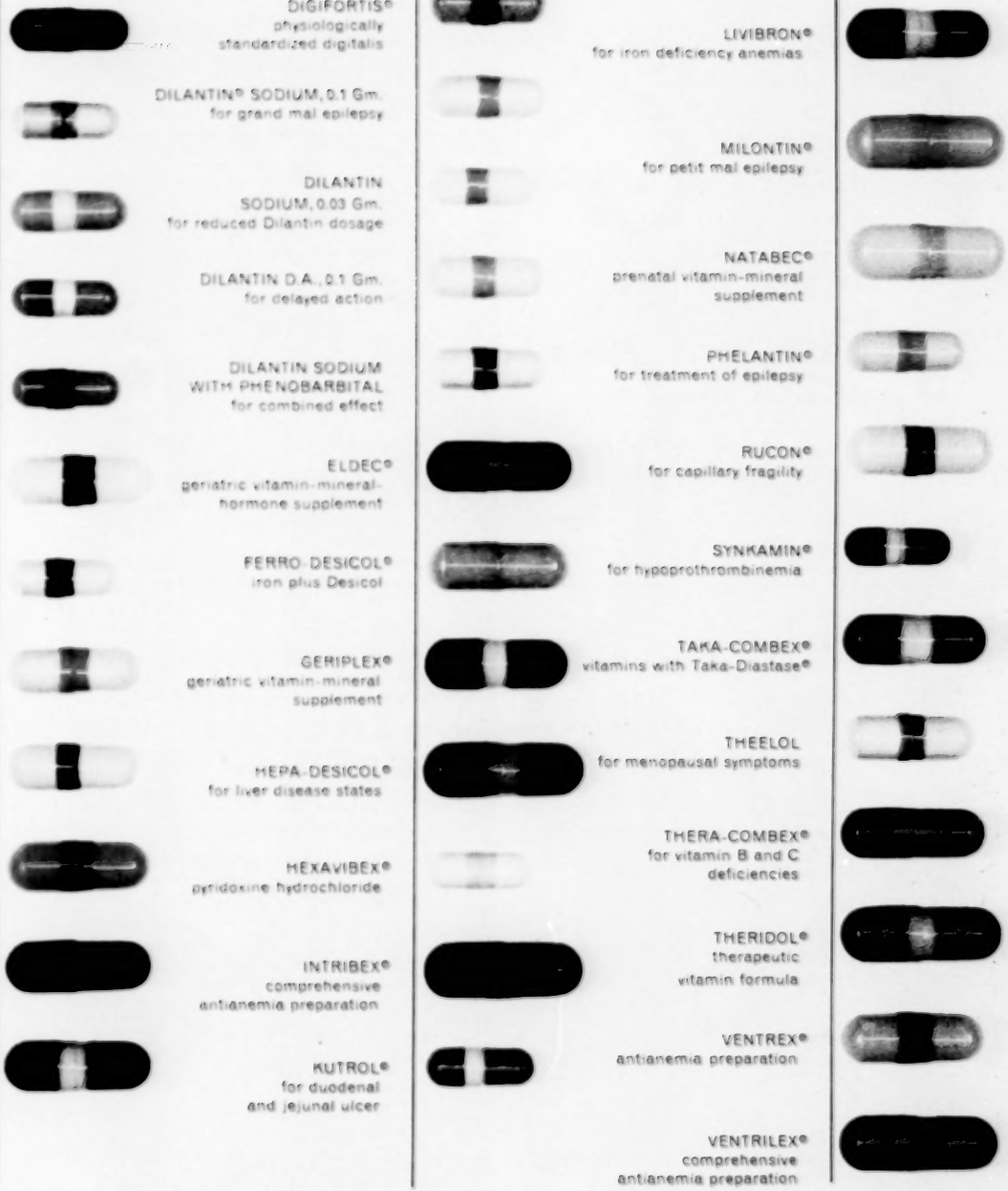
References: (1) Stein, I. D.: Circulation 12:833, 1955. (2) Potvin, L.: Bull. Assoc. méd. lang. franç. Canada 85:941, 1956. (3) Sigg, K.: Angiology 8:44, 1957. (4) Elder, H. H. A., and Armstrong, J. B.: Practitioner 178:479, 1957. (5) Braden, F. R.; Collins, C. G., and Sewell, J. W.: J. Louisiana M. Soc. 109:372, 1957.

07888

The distinctive colored bands identify

KAPSEALS®

as Parke-Davis products



ABDEC®
comprehensive multivitamin formula

AMBODRYL® HYDROCHLORIDE
antihistaminic

AMPHEDASE®
anorectic-antidepressant

BENADRYL® HYDROCHLORIDE
antihistaminic-antispasmodic

BENADRYL HYDROCHLORIDE WITH EPHEDRINE SULFATE
antihistaminic plus sympathomimetic

CARBRITAL®
dual-action hypnotic

CARBRITAL HALF-STRENGTH
for daytime sedation

CELONTIN®
controls petit mal and psychomotor attacks

CHLOROMYCETIN®
broad-spectrum antibiotic

CHLOROSTREP®
for enteric infections

COMBEX®
vitamin B formula

COMBEX WITH VITAMIN C
for vitamin B and C deficiencies

DESICOL®
desiccated whole bile

DIGIFORTIS®
physiologically standardized digitalis

DILANTIN® SODIUM, 0.1 Gm.
for grand mal epilepsy

DILANTIN SODIUM, 0.03 Gm.
for reduced Dilantin dosage

DILANTIN D.A., 0.1 Gm.
for delayed action

DILANTIN SODIUM WITH PHENOBARBITAL
for combined effect

ELDEC®
geriatric vitamin-mineral-hormone supplement

FERRO-DESICOL®
iron plus Desicol

GERIPLEX®
geriatric vitamin-mineral supplement

HEPA-DESICOL®
for liver disease states

HEXAVIBEX®
pyridoxine hydrochloride

INTRIBEX®
comprehensive antianemia preparation

KUTROL®
for duodenal and jejunal ulcer

LIVIBRON®
for iron deficiency anemias

MILONTIN®
for petit mal epilepsy

NATABEC®
prenatal vitamin-mineral supplement

PHELANTIN®
for treatment of epilepsy

RUCON®
for capillary fragility

SYNKAMIN®
for hypoprothrombinemia

TAKA-COMBEX®
vitamins with Taka-Diastase®

THEELOL
for menopausal symptoms

THERA-COMBEX®
for vitamin B and C deficiencies

THERIDOL®
therapeutic vitamin formula

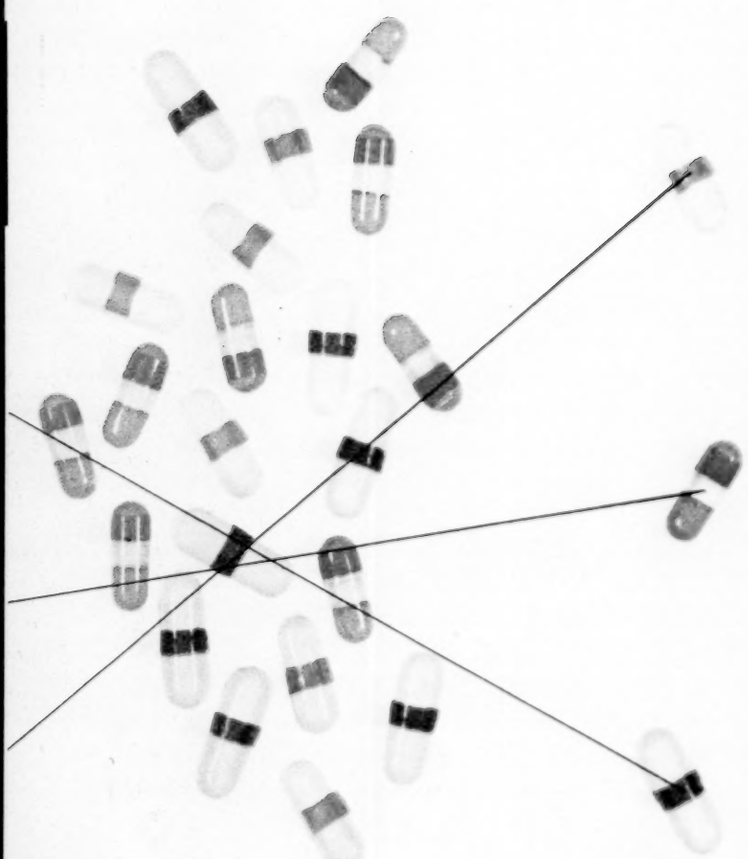
VENTREX®
antianemia preparation

VENTRILEX®
comprehensive antianemia preparation

MEDICAMENTA VERA

PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

WITH KAPSEALS®...IMMEDIATE IDENTIFICATION



Each KAPSEAL is identified as a Parke-Davis product by the unique color band.

no confusing these well-known Parke-Davis products...

DILANTIN® SODIUM
(diphenylhydantoin sodium, Parke-Davis)

A widely used anticonvulsant especially effective in the treatment of grand mal epilepsy, DILANTIN prevents or greatly decreases the incidence and severity of seizures. It has little hypnotic or narcotizing effect. DILANTIN may also be used in certain other convulsive states.

BENADRYL® Hydrochloride
(diphenhydramine hydrochloride, Parke-Davis)

BENADRYL provides rapid, dual-action relief of allergic symptoms. Its antihistaminic action promptly controls rhinorrhea, itching, nasal blocking, and related histamine reactions, while its atropine-like antispasmodic effect suppresses bronchial and gastrointestinal spasms.

CHLOROMYCETIN®
(chloramphenicol, Parke-Davis)

A highly effective broad-spectrum antibiotic, CHLOROMYCETIN gives excellent clinical results in many infectious disorders, including cutaneous and subcutaneous infections, ophthalmic infections, infections of the ear, nose, and throat, enteric and urogenital infections, infections of the central nervous system, generalized systemic infections, and surgical infections.

PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

P-765

FOR THE CARDIAC CANDIDATE CAUGHT IN THE PRESSURE SQUEEZE

AVOIDS THE THERAPEUTIC EXTREMES OF PREVIOUS RESERPINE AND RAUWOLFIA AGENTS

"DEPRESSION ACCOMPANIED BY ANXIETY...WAS NOT SEEN..."[1] WITH THIS NEW ALKALOID OF RAUWOLFIA. OTHER UNDESIRABLE RAUWOLFIA-RESERPINE REACTIONS ARE SELDOM ENCOUNTERED OR ARE MINOR IN DEGREE.[1-3] PRODUCES SIGNIFICANTLY MORE STABLE AND SUSTAINED CONTROL OF THE TENSION-HYPERTENSION COMPLEX.[4] HIGHER DOSAGES MAY BE EMPLOYED WHERE INDICATED, WITH CONTINUED EXCELLENT TOLERATION.[1-4] ALSO PREFERRED FOR THERAPY OF ACUTE ANXIETY STATES AND CHRONIC MENTAL DISORDERS.[4,5]

Supplied: MODERIL Tablets—yellow, scored 0.25 mg. oval tablets, bottles of 100 and 500; salmon, scored 0.5 mg. oval tablets, bottles of 100.

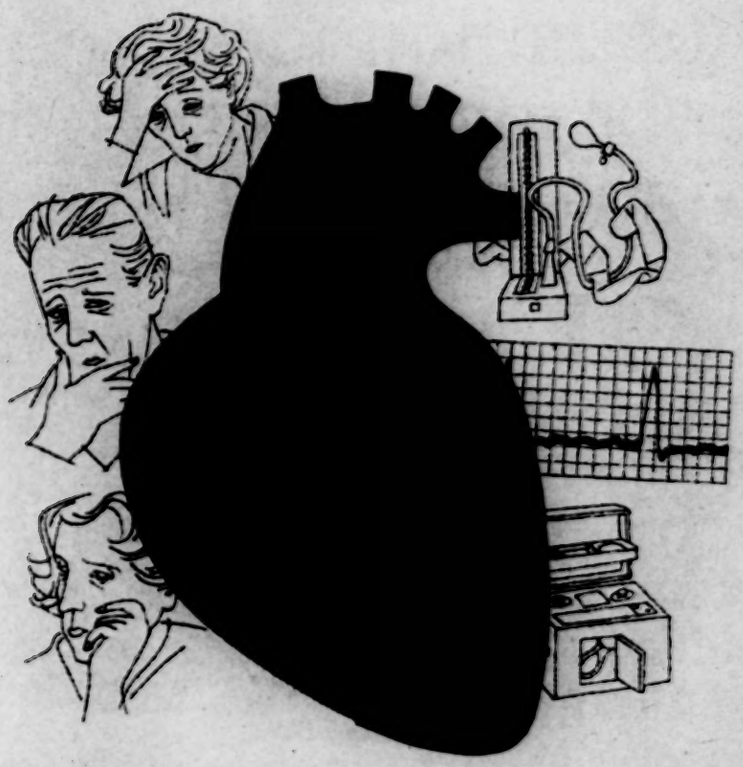
Dosage: The recommended initial dosage is 0.5 mg. twice daily for two weeks, with reduction thereafter to a minimum maintenance dosage of 0.25 mg. once daily. For greater hypotensive effect after initial period, increase dosage cautiously by 0.25 mg. daily up to a maximum daily dosage of 2.0 mg. Prescribe after meals.

1 Moyer, J. H., et al.: A.M.A. Arch. Int. Med. 96:530, 1955. 2. Moyer, J. H., et al.: South. M. J. 50:499, 1957. 3. Smirk, F. H., and McQueen, E. G.: Lancet 2:115, 1955. 4. Winton, S. S.: Internat. Rec. Med. 170:665, 1957. 5 Malamud, W., et al.: Am. J Psychiat 114:193,1957.

Pfizer

PFIZER LABORATORIES
Division,
Chas. Pfizer & Co., Inc.
Brooklyn 6, New York

angina attacks



Miltrate*

MILTOWN® + PETN

The long-acting nitrate, PETN, helps maintain normal myocardial metabolism while Miltown relieves fear, anxiety and tension. As a result, Miltrate controls both physical and emotional causes of angina attacks.

Miltrate increases exercise tolerance

- reduces nitroglycerin dependence
- is notably safe for prolonged use
- provides convenient one-tablet dosage

Supplied: Bottles of 50 tablets.

Each tablet contains: 200 mg. Miltown + 10 mg. pentaerythritol tetranitrate.

Usual dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime. Dosage should be individualized.

References:

1. Shapiro, S.: Observations on the use of meprobamate in cardiovascular disorders. Angiology 8:504, Dec. 1957.
2. Friedlander, H. S.: The role of ataraxics in cardiology. Am. J. Cardiol. 1:395, March 1958.
3. Eskuith, I. S.: The holistic approach to angina pectoris. Am. Heart J. 55:621, April 1958.

WALLACE LABORATORIES, *New Brunswick, N. J.*

*TRADE-MARK

CHL-8837-19

Squibb announces a new, topically superior anti-inflammatory steroid

Kenalog-S

Squibb Triamcinolone Acetonide with Neomycin and Gramicidin (Spectrocin)

Lotion

with added antibacterial protection

Kenalog-S is a new, clinically superior topical corticoid lotion with added protection against bacterial infection to rapidly relieve itching, inflamed or infected skin lesions.

1. Provides effective anti-inflammatory, antipruritic, antiallergic action for rapid symptomatic relief.

2. Produces clinical results superior to hydrocortisone.

3. Extremely well tolerated—no edema, no electrolyte imbalance or systemic toxicity.

4. 92% effective . . . typical of clinical reports, Kenalog-S Lotion proved 92% effective in 67 patients with various dermatologic disorders.*

5. Attacks pyogenic infections . . . protects against secondary infections . . . with the proved antibacterial action of Spectrocin (neomycin and gramicidin).

6. The consistently effective results and the excellent spreading ability permits supplying of Kenalog-S Lotion in 7.5 cc. economy packages.

and a companion to Kenalog-S

Kenalog Triamcinolone Acetonide **for topical application in dermatotherapy**

Effectively provides prompt symptomatic relief as well as control of most common dermatologic disorders.

Kenalog Cream, 0.1%—5 Gm. and 15 Gm. tubes
Kenalog Lotion, 0.1%—15 cc. plastic squeeze bottles
Kenalog Ointment, 0.1%—5 Gm. and 15 Gm. tubes

*Monographs on Therapy 3:137 (Nov.) 1958

SQUIBB *Squibb Quality—the Priceless Ingredient.*

in Egypt,
it's called 'Gippy-tummy'
(EGYPTIAN STOMACH)

diarrhea by any name

GASTROENTERITIS
BACILLARY DYSENTERY
PARADYSENTERY
SALMONELLOSIS
DIARRHEA OF THE NEWBORN
NONSPECIFIC DIARRHEA
"SUMMER COMPLAINT"

usually responds rapidly to

Cremomycin®

NEOMYCIN-SULFASUXIDINE-KAOLIN-PECTIN SUSPENSION

for *rapid* relief of all diarrheas—regardless of etiology

fruit-flavored, readily accepted by patients of all ages*

Neomycin—rapidly bactericidal against most intestinal pathogens, but is relatively ineffective against such diarrhea-causing organisms as Shigella.

Sulfasuxidine—an ideal adjunct to neomycin because it is highly effective against Shigella and certain other neomycin-resistant organisms.

Kaolin and Pectin—coat and soothe the inflamed mucosa, absorb toxins, help reduce intestinal hypermotility, help provide rapid symptomatic relief.

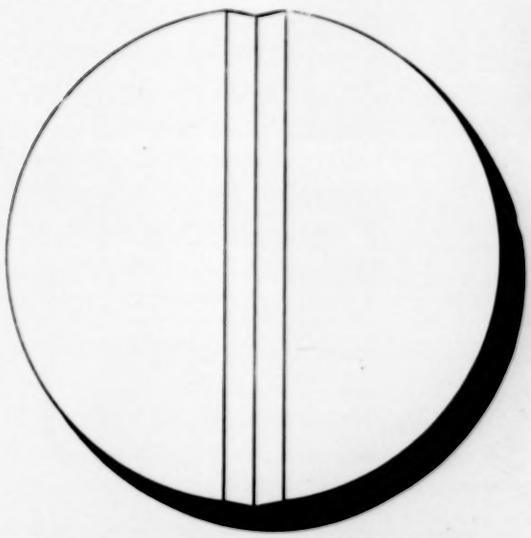
***For infants, CREMOMYCIN may be administered in the regular bottle feeding, since its fine particles easily pass through a standard nursing nipple.**

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILA. 1, PA.

GREATER CONVENIENCE

DOUBLE POTENCY

AT LOW COST TO YOUR PATIENT



Pentids '400'

Squibb 400,000 units Buffered Penicillin G Potassium Tablets

For the treatment of penicillin susceptible infections—ranging from **mild to moderately severe**—due to **hemolytic streptococcus / pneumococcus / staphylococcus / and for the prevention of streptococcal infections where there is a history of rheumatic fever**

Clinical effectiveness confirmed by millions of cases
Specific in many common infections
Daily dosage may be spaced without regard to mealtime
Ease of administration with oral penicillin
Economy for the patient

SQUIBB

Squibb Quality— the Priceless Ingredient

new convenient oral tablets

PENTIDS '400,' each scored tablet contains 400,000 units of penicillin G potassium buffered, bottles of 12 and 100. Twice the unitage of Pentids 200,000 units.

also available

PENTIDS, 200,000 units of buffered penicillin G potassium per each scored tablet, bottles of 12 and 100, and 500.

PENTIDS FOR SYRUP, 200,000 units of penicillin G potassium per teaspoonful (5 cc.), 12 dose bottles.

PENTIDS CAPSULES, 200,000 units of penicillin G potassium per capsule, bottles of 24 and 100, and 500.

PENTIDS SOLUBLE TABLETS, 200,000 units of penicillin G potassium per tablet, vials of 12 and bottles of 100.

PENTIDS-SULFAS TABLETS, 200,000 units of penicillin G potassium with 0.5 Gm. triple sulfas per tablet, bottles of 30 and 100, and 500.

PENTIDS® IS A SQUIBB TRADEMARK

HELPS PALSIED PATIENTS "LIVE AGAIN"

COGENTIN®

METHANESULFONATE (BENZTROPINE METHANESULFONATE)

rated the best single drug for the palsied patient[1]

- Well tolerated and markedly effective, COGENTIN *"should be added to the treatment program of every patient with paralysis agitans."*[2]
- COGENTIN *gives symptomatic relief* in all types of parkinsonism—whether postencephalitic, idiopathic, or arteriosclerotic.
- COGENTIN *provides highly selective action* such as no other current drug affords.[2] It is often of benefit in rigidity, muscle spasm, even in severe tremor.[3] The contracture of parkinsonism is relieved and posture is improved.[3]
- *With the help of* COGENTIN, *therapy with tranquilizers can often be continued* in patients in whom trembling would otherwise force reduction or withdrawal.[4]

As COGENTIN is long-acting, one dose daily may be sufficient.

Supplied: as 2 mg. quarter-scored tablets in bottles of 100 and 1000.

1. M. Clin. North America 38:485 (March) 1954. 2. J.A.M.A. 162:1081, 1956. 3. J.A.M.A. 156:680, 1954. 4. Yale J. Biol. & Med. 28:308, 1955/56.

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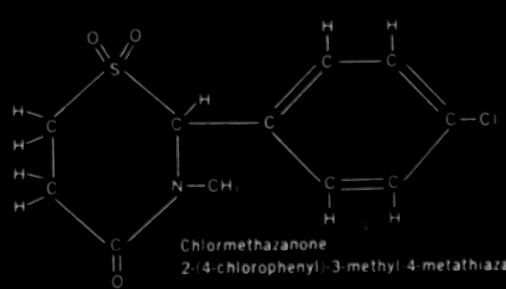

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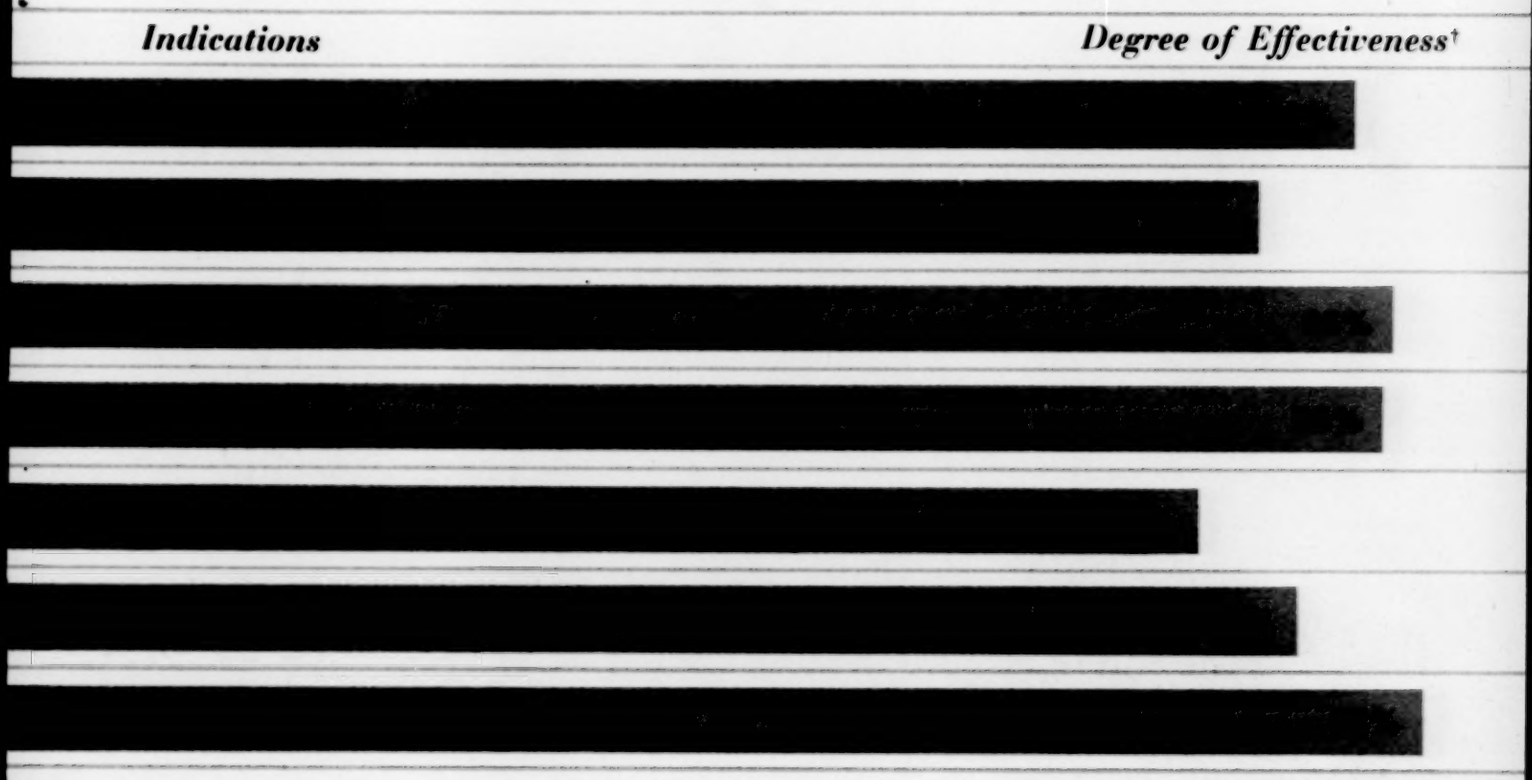

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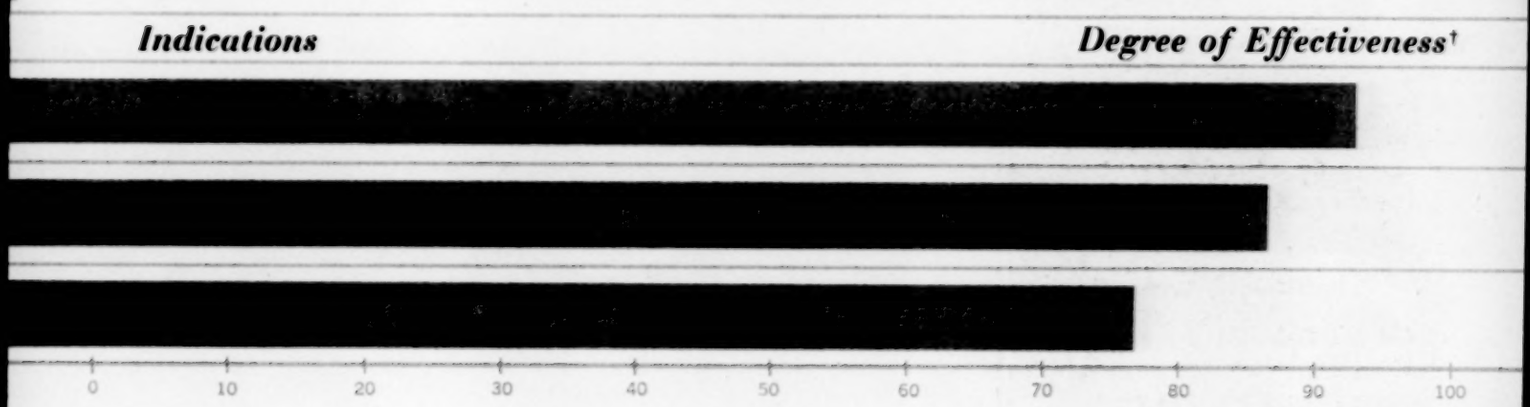

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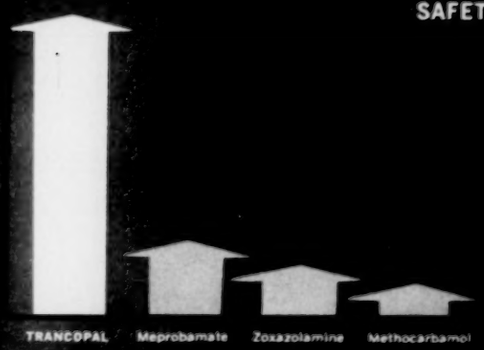

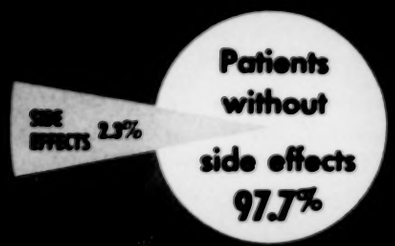

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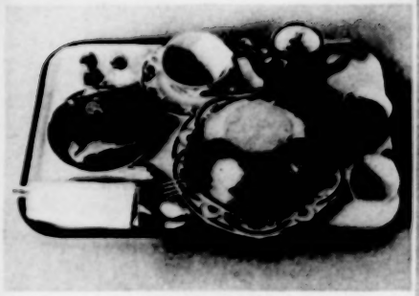

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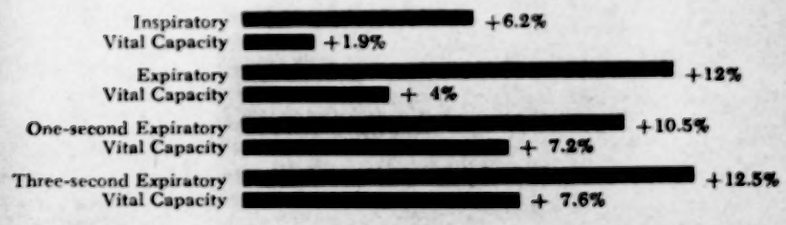

*Adapted from Leslie, A., and Simmons, D. H.[3]

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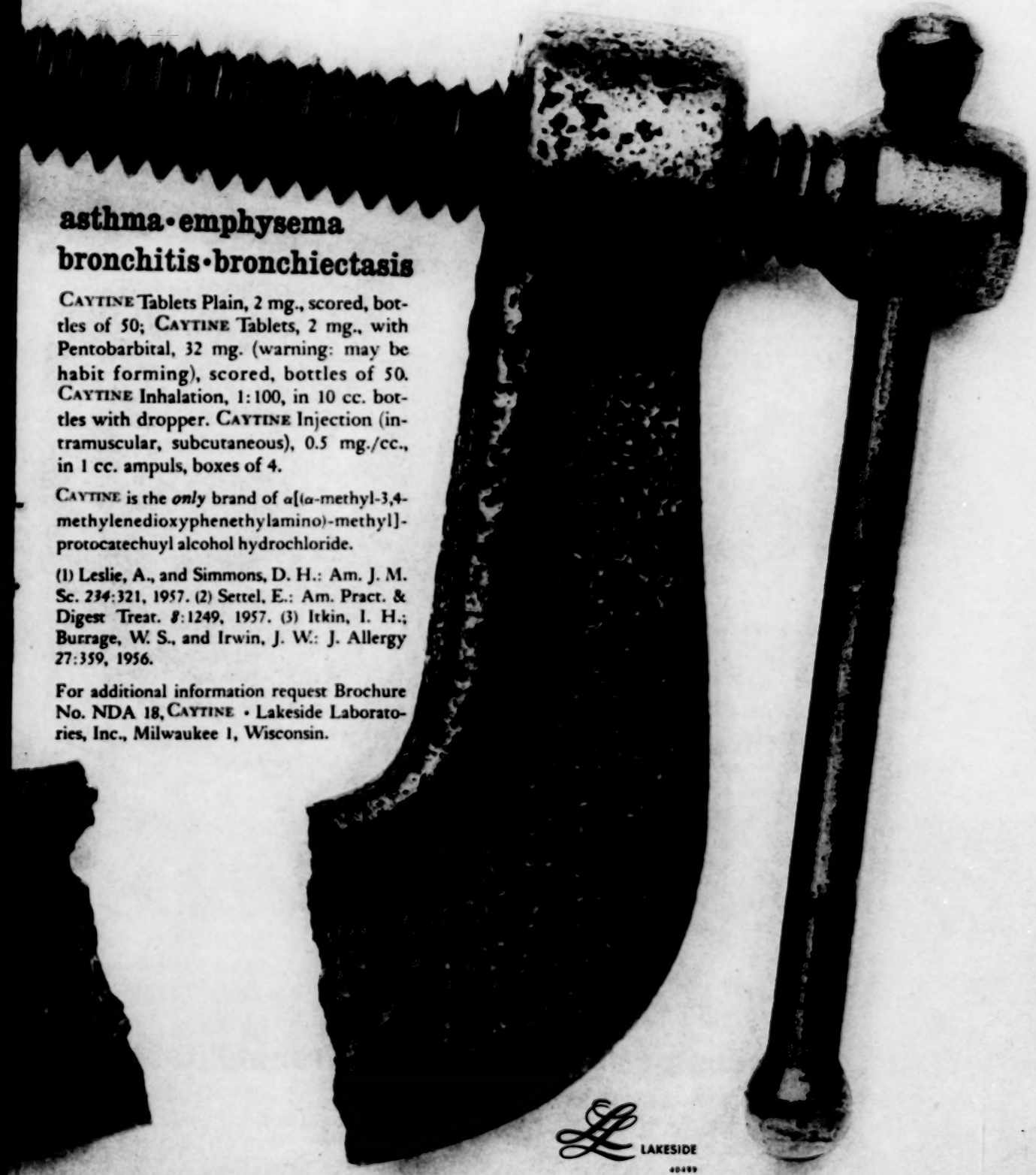

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(1) Leslie, A., and Simmons, D. H.: Am. J. M. Sc. *234*:321, 1957. (2) Settel, E.: Am. Pract. & Digest Treat. *8*:1249, 1957. (3) Itkin, I. H.; Burrage, W. S., and Irwin, J. W.: J. Allergy *27*:359, 1956.

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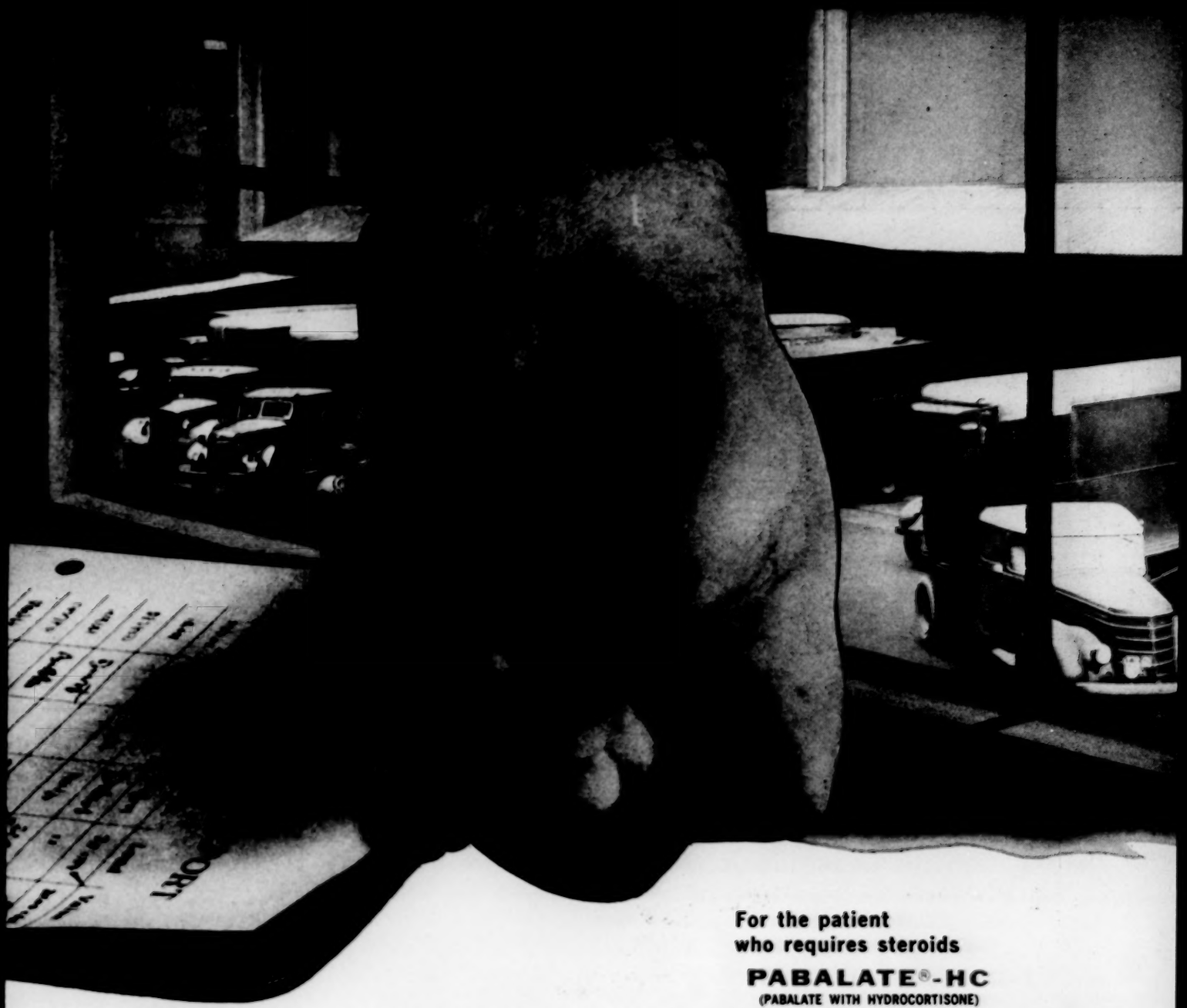

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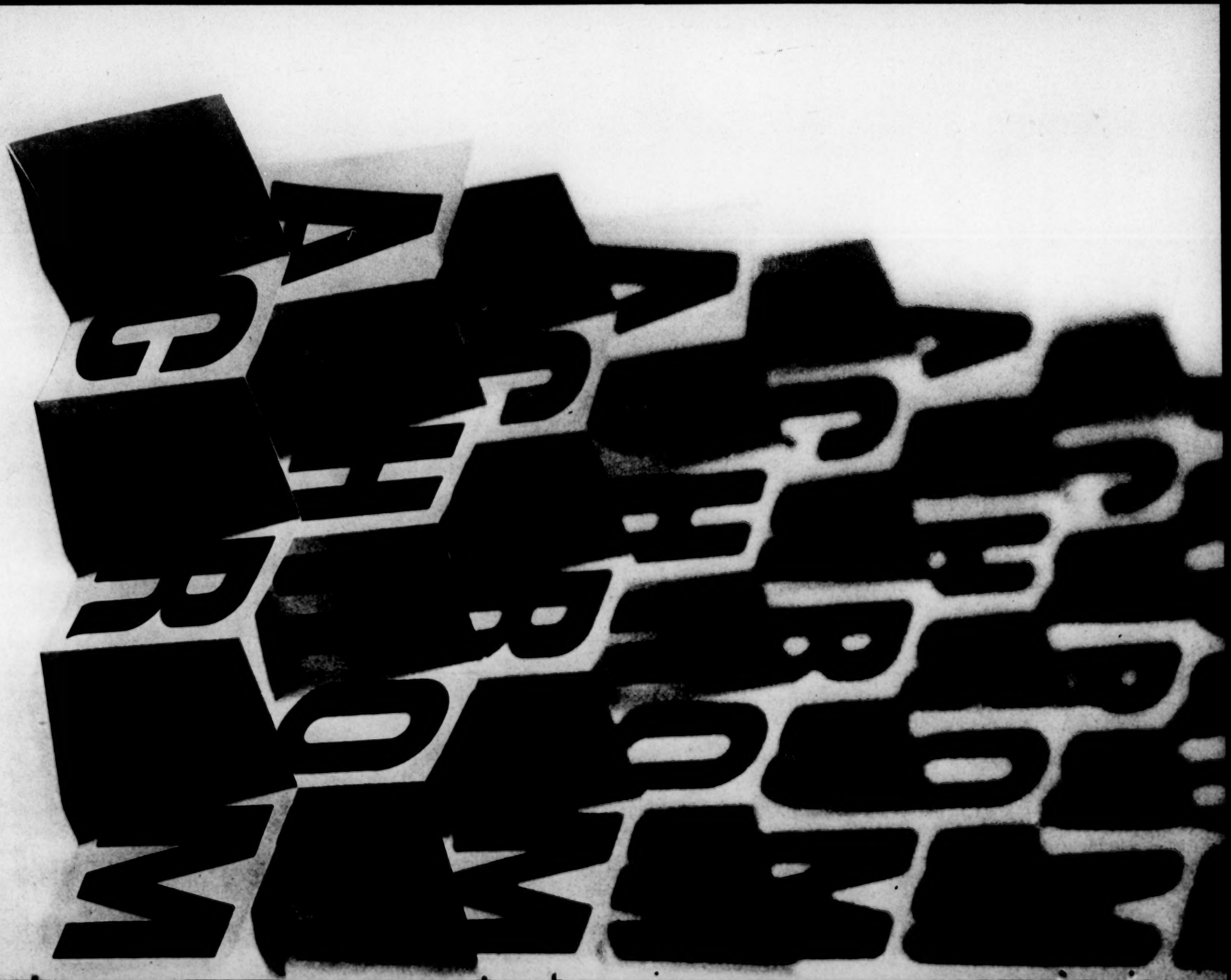

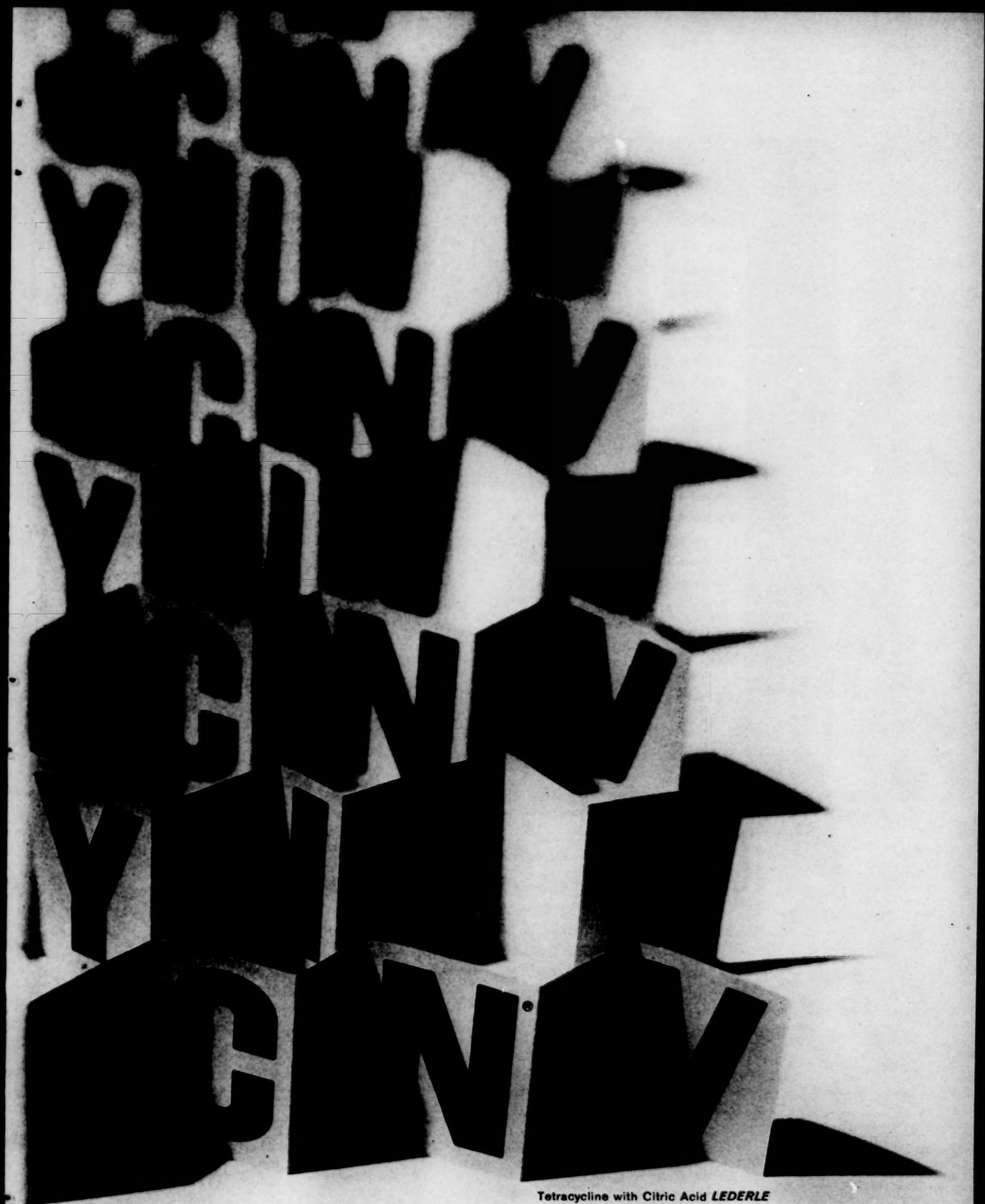

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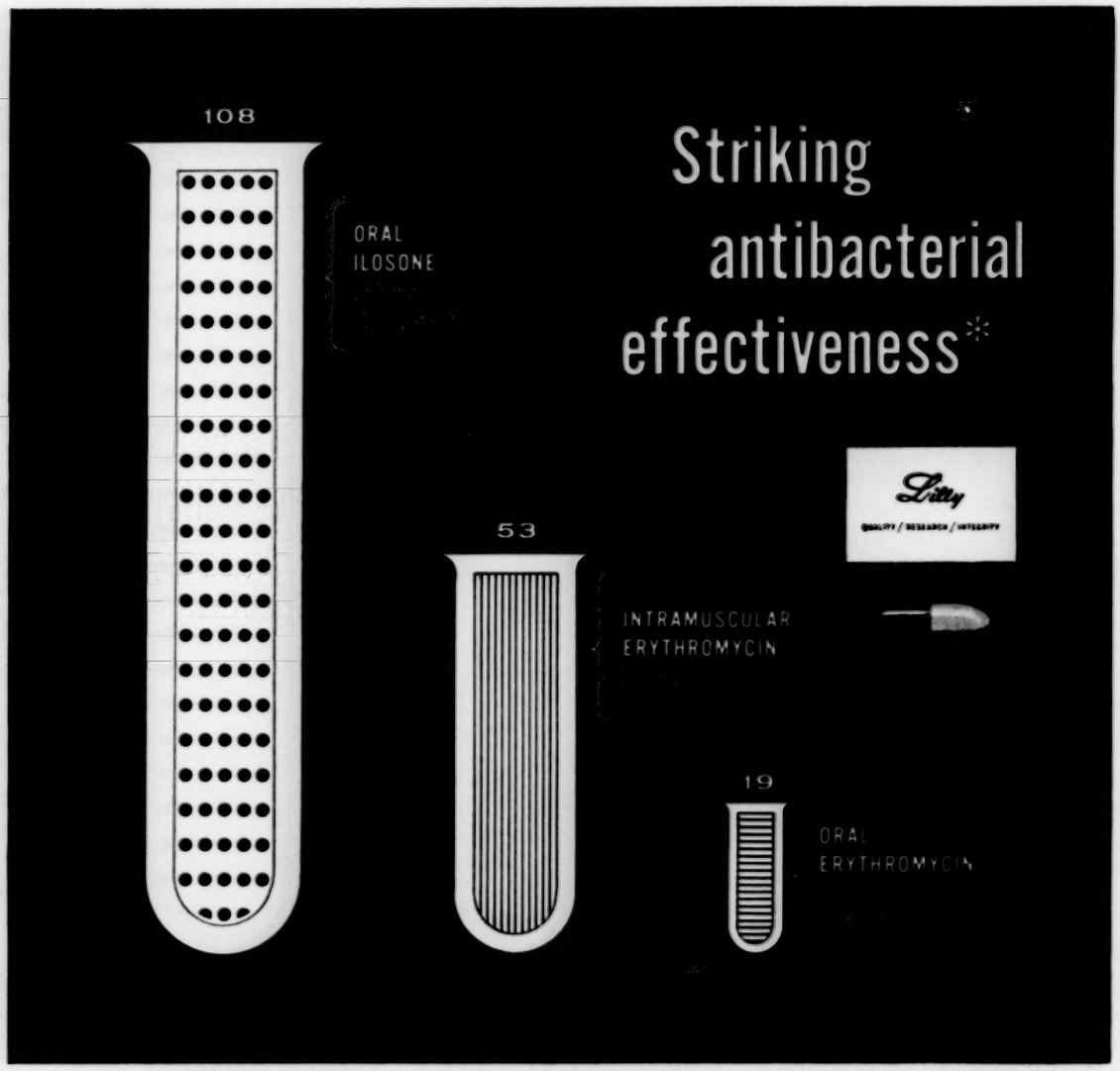

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1. Griffith, R. S., *et al.*: Antibiotic Med. & Clin. Therapy, *5*:609 (October), 1958. Note: Peak levels with the oral erythromycin tablets (thirty-three dilutions) were not observed until four hours after administration. 2. Data from Griffith, R. S.: Antibiotics Annual, p. 269, 1954-1955.

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The American Journal of Medicine

VOL. XXVI FEBRUARY, 1959 NO. 2

Editorial

Symmetry, Asymmetry and Meso-Symmetry

SYMMETRY is so ordinary a concept that one rarely bothers to explore it carefully. We readily appreciate the superficial symmetry of the human body, recognizing that it conceals a highly asymmetrical distribution, not only of organs but also of functions. In view of the apparent absence of any subtlety in the "common sense" ideas about symmetry and asymmetry, it is interesting that very fundamental discoveries relating to these concepts continue to be made.

A solid is said to possess a plane of symmetry if a plane may be passed through it dividing it into two halves such that the one is the mirror image of the other. Superficially this condition is at least approximately fulfilled by the body of a man, provided he is either bald or parts his hair in the middle. Although man may exhibit some degree of symmetry, his right hand exhibits none. It is because of this that we have no difficulty in recognizing it as a right hand, even though it be amputated. Yet the right hand bears an obvious and specific relation to the left hand. The one is a mirror image of the other. Two tennis balls, or two like cubes, are also mirror images of each other, but in contrast to a pair of hands, these pairs are also superimposable and themselves exhibit symmetry. The right and left hands, mirror images but not superimposable, are said to be *antipodes*.

The occurrence of chemical antipodes was clearly recognized by Pasteur [*1*] who showed that certain tartrates, under certain conditions, formed crystal mixtures which could be separated into two antipodal forms. The shapes of the two species of crystals were, like the right and left hands, mirror images of each other but not superimposable. Of the greatest importance to chemistry was his associated observation that the crystals of one form gave solutions which rotated the plane of polarized light to the right (*d* = dextro-), whereas a solution of crystals of the antipodal form rotated the plane of polarized light by the same amount, but to the left (*l* = levo-). Simultaneously and independently, Le Bel [*2*] and van't Hoff [*3*] arrived at the correct explanation of this asymmetry, basing their views on the assumption that the four valence bonds of carbon are directed toward the apices of a symmetrical tetrahedron. It could readily be shown that in any compound in which one carbon atom bears four different substituents (*Cabde*), two configurations are possible. (Fig. 1.) Each of these will be devoid of a plane of symmetry, they will be mirror images of each other, but they will be non-superimposable. A solution of either species of molecule will be optically active, i.e., it will rotate the plane of polarized light, and the crystals of the two species may

[1] PASTEUR, L. Réchèrches sur les relations qui peuvent exister entre la forme crystalline, la composition chimique et le sens de la polarization rotatoire. *Compt. rend.*, 26: 535, 1848.

[2] LE BEL, J. A. Sur les relations qui existent entre les formules antomique des corps organique et le pouvoir rotatoire de leur dissolutions. *Bull. Soc. Chim.*, 22: 337, 1874.

[3] VAN'T HOFF, J. H. A treatise on a system of atomic formulae in three dimensions and on the relation between rotatory power and chemical constitution, 1874. Cited by Lowry, T. M. Optical Rotatory Power, p. 42. Edited by Donnan, F. G. London, 1935. Longmans, Green & Co.

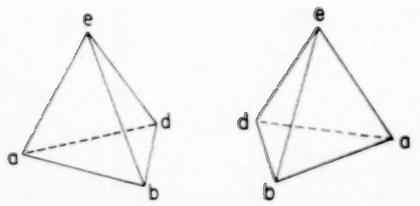


FIG. 1. Antipodal forms of the type *Cabde* [2,3]. In this and subsequent figures, the carbon atom, C, is pictured as residing at the center of a tetrahedron. The two tetrahedra are mirror images but not superimposable. Neither has a plane of symmetry.

reflect the antipodal molecular forms and be themselves antipodal.

This hypothesis, with a number of correlaries of a relatively minor nature, has survived and has become a sheet-anchor of structural and configurational organic chemistry. The tetrahedral distribution of the four valences of carbon and the consequences thereof relating to asymmetry and optical activity have become basic articles of faith of the organic chemist, and predictions based upon these principles have been found to be fulfilled in the laboratory in the vast majority of cases. Few generalizations in the entire history of science have had such a gratifying development.

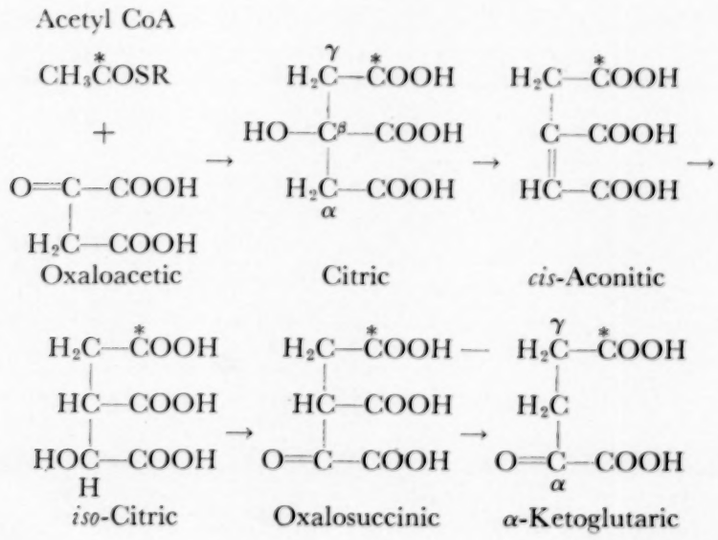
Studies of the citric acid cycle in a number of laboratories led to what was initially a puzzling conclusion. Citric acid was known to arise from the condensation of a two-carbon and a four-carbon fragment, acetyl coenzyme A and oxaloacetic acid respectively, and its further metabolism led, over a series of steps to α-ketoglutaric acid:

The puzzle stemmed from the experimental finding that an isotopic label introduced into the carboxyl carbon of acetyl (*) showed up exclusively in the γ-carboxyl of α-ketoglutarate. Yet in the intermediate citric acid, the α- and γ-carboxyls were symmetrically disposed and, it was argued, a label introduced into either of these positions would in effect be equally distributed in both positions.

This apparent paradox was resolved by the suggestion of Ogston [4] that although the citric acid molecule was symmetrical, if it attached to an asymmetric enzyme surface, aconitase in the present instance, by no less than three points of attachment, it would behave asymmetrically. The situation may be visualized from Figure 2 wherein the central (β) carbon of citric acid is considered to reside at the center of a tetrahedron. The four substituents are distributed at the apices of this tetrahedron and the resulting figure possesses a plane of symmetry. Three-point attachment may now be pictured as attachment to a face of this tetrahedron. Study of the figure will reveal that no two of the four triangular faces of this tetrahedron are identical, and that on an asymmetric enzyme surface which requires attachment at three different points (*a*, *b* and *d*), one and only one face is accommodated. Thus the enzyme clearly distinguishes between the two —CH_2COOH (*a*) substituents even though these are symmetrically disposed about the central carbon atom.

A more generalized resolution of the problem

[4] OGSTON, A. G. Interpretation of experiments on metabolic processes, using isotopic tracer elements. *Nature, London*, 162: 963, 1948.



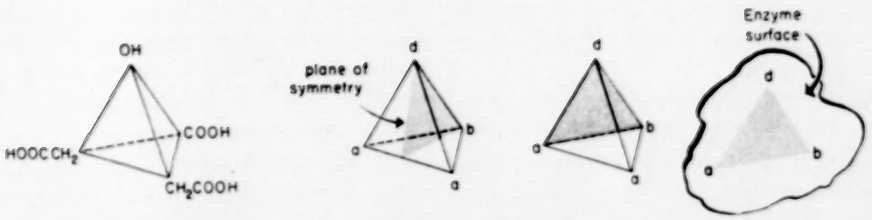


FIG. 2. Symmetry of citric acid [4]. Citric acid, a compound of the class *Caabd*, has a plane of symmetry. Despite this fact, if it reacts with an asymmetric enzyme after attachment at no less than three points (*abd*), only one face of the tetrahedron (shaded) can be accommodated on the enzyme and discrimination between the two $-CH_2COOH$ (*a*) groups will result.

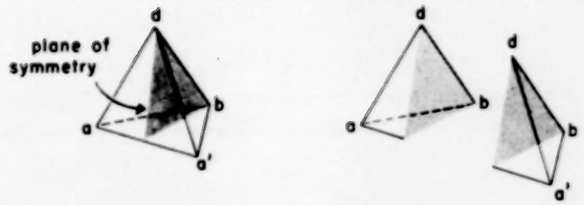
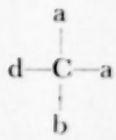


FIG. 3. The *meso*-carbon atom [5]. A carbon atom substituted by two like and two unlike substituents, *Caabd*, when split along its plane of symmetry generates two halves which are not identical. These halves are non-superimposable mirror images of each other. The two *a* substituents, designated *a* and *a'*, are antipodal to each other.

has been proposed by Schwartz and Carter [5]. These authors point out that there are certain peculiarities to the class of organic compounds which bear two like and two unlike substituents on a carbon atom,



of which citric acid is but one of many possible examples. To a carbon atom in this situation (*Caabd*) the name *meso*-carbon has been given. This molecule undoubtedly has a plane of symmetry, as defined in the second paragraph. However, *the two half-molecules which would result from cleavage along this plane of symmetry, while mirror images of each other, are not identical.* To be identical, these two half-molecules would necessarily have to be in addition superimposable, and this condition they do not fulfill. The two half-molecules about a *meso*-carbon atom are each in effect asymmetric and are antipodes of each other.

[5] SCHWARTZ, P. and CARTER, H. E. A nonenzymatic illustration of "citric acid type" asymmetry: the meso-carbon atom. *Proc. Nat. Acad. Sc.*, 40: 499, 1954.

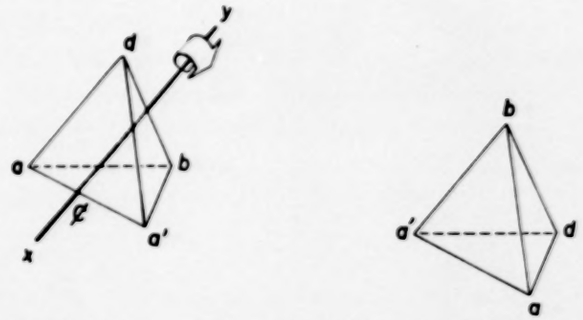


FIG. 4. The *meso*-carbon atom [5]. The three-dimensional representation of *Caa'bd* is rotated 180° about axis *xy* so that the initial positions of *a* and *a'* have been reversed. The resultant picture is not superimposable upon the initial picture.

As a result of this situation, the two *a* substituents in a compound of the type *Caabd*, previously regarded as identical and indistinguishable, are now seen to be non-identical and may be labeled *a* and *a'*. The distinction between these two substituents may be appreciated if we consider the triangular face of the tetrahedron (Fig. 3) which each *a* substituent sees opposite itself. Substituent *a'* sees a triangle *a d b*, moving clockwise, whereas *a* sees a triangle *a' b d*. This subtle difference is of no consequence when a symmetrical reagent is offered the option of attacking *a* or *a'*. An unsymmetrical reagent, however, whether an enzyme or a simpler organic molecule, will see in *a* and *a'* two different sites, and will attack them with differing facility.

The distinction between the Ogston hypothesis and that of Schwartz and Carter is fine but nonetheless real. In Ogston's view, substituents *a* and *a'* in a molecule *Caa'bd* are identical and become non-identical only after the molecule is attached by three or more points to an asymmetric surface, such as an enzyme. The novel point made by Schwartz and Carter is that these two *a* substituents differ from each other *ab initio*, even when the molecule is unattached and free-

swimming. Attack of *Caa'bd* by an asymmetric reagent does not generate this difference, it merely makes it evident.

An alternative and more general treatment of the *meso*-carbon atom has been proposed by Hirschmann [6]. He suggests that a three dimensional representation of a molecule containing two like substituents, *a* and *a'*, be so moved about that *a* occupy the location formerly occupied by *a'* and *vice versa*. If the resultant picture is not superimposable upon the initial picture (Fig. 4), an asymmetric reagent will discriminate between *a* and *a'*.

Reverting to our analogy of a man, with his superficial symmetry, we can recognize now that either by the test of Schwartz and Carter or by that of Hirschmann, this symmetry is of the *meso* variety. Symmetrical tools, such as a tennis racket, a spoon or hammer are handled with equal ease by a right-handed or left-handed man. Unsymmetrical tools, such as a golf club or a Van Slyke gas analysis apparatus, present special problems to a left-handed man. The former he resolves by ordering an antipodal set of golf clubs. Left-handed Van Slyke apparatuses not being commercially available, the left-handed man is forced to solve this problem as best he can. It is a matter of passing interest that the problem of *meso*-symmetry has been faced and solved by the sporting goods manufacturer years ago but that only very recently has it inpinged on the consciousness of the chemist.

DeWitt Stetten, Jr., M.D.
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

[6] Hirschmann, H. The structural basis for the differentiation of identical groups in asymmetric reactions. In: Essays in Biochemistry. p. 156. Edited by Graff, S. New York, 1956. John Wiley & Sons, Inc.

Clinical Studies

Quantitative Relationship between Insulin Dosage and Amount of Carbohydrates Utilized in Diabetic Persons*

MICHAEL SOMOGYI, PH.D. *and* H. V. GOLDWASSER, M.D.†
St. Louis, Missouri

ESTIMATION of the insulin requirement of diabetic patients has to this day remained a purely empirical procedure, with the goal to eliminate glycosuria and restore normal blood sugar levels. When insulin became available for clinical use, attainment of this goal appeared to be simple enough: since—according to the generally accepted theory—diabetes is due to a deficient insulin supply (hypoinsulinism), treatment with insulin was regarded as simple substitution therapy. The height of abnormal hyperglycemia and the extent of glycosuria are —it was believed—in direct quantitative relation to the degree of the insulin deficiency, hence the amount of extraneous insulin to be administered can be calculated on a stoichiometric basis. Clinical experiments seemed to prove the validity of this concept. As early as 1922 Wilder et al. [*1*] published results of clinical experiments with diabetic adults showing, by elaborate computations, that each unit of injected insulin "metabolizes" on the average 1.59 gm. of glucose. Soon afterwards Hartmann [*2*] found much the same G/I ratio (G = grams of glucose utilized, I = units of insulin injected) in experiments with diabetic children.

Shortly thereafter, however, a number of other authors derived very different figures from their own experiments. Williams [*3*], for instance, presented an equation with a G/I ratio of 4.0 instead of 1.6 and claimed consistent success for its application in clinical practice. Holm [*4*] tried to find a reliable G/I ratio in experiments with depancreatized dogs which were administered glucose and insulin intravenously, at constant rates. His results indicated 8.75 gm. as the amount of glucose that is utilized by the action of each unit of insulin. But Holm seems to have overlooked an important piece of earlier work by Allan [*5,6*] who, in a search for an ideal tool for the bioassay of insulin preparations, turned to depancreatized dogs as a medium in which, interference of endogenous insulin being ruled out, utilization of glucose could be fully credited to the injected insulin. In the depancreatized dog, he assumed, insulin action could be accurately titrated, so to speak, against glucose. But the results of the ably executed experiments crassly contradicted this assumption: instead of yielding a G/I ratio even remotely approaching a constant value, 1 unit of insulin helped to utilize from as little as 0.8 gm. all the way to 22 gm. of glucose. Allan's data clearly showed that the magnitude of the G/I ratio depended on the quantitative relationship between the amount of glucose fed and the amount of insulin injected: the G/I ratio increased as the amount of glucose was raised and the insulin dosage kept constant; and it decreased when the insulin dosage was increased and a constant amount of glucose was administered.

Subsequently a variety of G/I ratios continued to crop up in the literature, their authors disregarding (or being unaware of) Allan's well controlled, reliable observations. Each author claimed validity for his own contention, but no one furnished any explanation of the bewildering discrepancies between his figure and those of other authors. The general confusion arising from this situation is fairly reflected in edition after edition of the widely known book of Joslin and his associates [*7*], when they raise the ques-

* From the Jewish Hospital of St. Louis, St. Louis, Missouri.
† Deceased.

TABLE I
SHOWING WIDE VARIATIONS IN THE AMOUNT OF CARBOHYDRATES UTILIZED PER UNIT OF INJECTED INSULIN (PATIENT M. K.)

Date	Diet (gm.)			Glucose (gm.)			Insulin (units)	G/I* Ratio
	P	F	CHO	Total Available	Lost in Urine	Utilized		
4/5/30	100	150	110	183	48	135	120	1.1
4/6/30	100	150	110	183	15	168	180	0.9
4/7/30	50	120	25	66	22	44	130	0.3
4/8/30	50	120	25	66	14	52	85	0.6
4/12/30	50	120	80	121	16	105	90	1.2
4/15/30	50	120	80	121	11	110	120	0.9
4/18/30	50	120	80	121	3	118	70	1.7
5/3/30	50	120	80	121	17	104	40	2.6

$$*\,G/I = \frac{\text{Grams glucose utilized}}{\text{Units insulin injected}}.$$

TABLE II
SHOWING VARIATIONS IN BLOOD SUGAR LEVEL IN INSULIN-TREATED DIABETIC (PATIENT M. K.)

Date	Time	Mg. Glucose* per 100 cc. Venous Blood
Insulin dosage: 70 units per day (40-10-20)		
4/16/30	7:00 A.M.	317
	9:45 A.M.	152
	11:15 A.M.	30
	1:15 P.M.	37
	4:00 P.M.	46
	5:45 P.M.	91
4/18/30	7:30 A.M.	296
	2:00 P.M.	65
4/23/30	7:40 A.M.	266
	1:45 P.M.	38

* True (fermentable) sugar.

tion: "How many grams of carbohydrate will 1 unit of insulin metabolize?" and answer it as follows: "In general 1 unit of insulin will metabolize 1 or 2 grams of carbohydrate, and even 3 to 6 grams; Newburgh says 7, and Holm even 8.75. . . . When we read that for each gram of glucose . . . 1 unit of insulin should be injected, we shrug our shoulders and exclaim—*Prenez garde!*" (They seem to have overlooked Allan's 22 gm.)

This problem inevitably confronted us in the routine of our hospital, and it became increasingly perplexing and acute as, in an effort to shed some light on the problem, we tried to support the clinical observations by greatly expanding the quantitative laboratory tests. The first such study, in which a thirty year old, "unmanageable" patient, M. K., served as subject, dates back to 1930. Following the generally accepted clinical routine, elimination of excessive hyperglycemia and, with it, abolition of glycosuria were set as the goal of insulin therapy. As long as hyperglycemia and substantial glycosuria persisted, the insulin dosage was regarded as insufficient and hence it was gradually increased. This procedure, however, proved to be futile. Increase of the insulin dosage would lead to mitigation or occasionally to the complete suppression of glycosuria, but only for brief periods, soon to yield to the recurrence of glycosuria, often in a much more severe degree than previously with a smaller insulin dose. Then the insulin dosage was increased again and, as before, without success, until mounting frequency and grievous severity of hypoglycemic reactions set a limit to further increase and even enforced a retreat in the dosage. The procedure was beset with panic and frustration. An excerpt from the voluminous protocols of these observations is presented in Table I. As may be noted, the most conspicuous feature of our findings is the absence of any direct proportionality in the quantitative relationship between insulin dosage and glucose utilized: The G/I ratio showed a scatter in the range of 0.3 to 2.6, i.e., it was nine times as great on one day as on another.

Efforts to establish normal or near normal blood sugar levels were equally futile. As may be seen in Table II, intolerably high hyperglycemias occurred in the morning before breakfast and after protracted hypoglycemic intervals during the day; and, it may be added, excessively high fasting blood sugars appeared even after severe hypoglycemic shocks, reported by the nurses to have taken place between 10 P.M. and midnight (blood samples for analysis were not taken at night).

At first we were inclined to regard the erratic response of this patient to insulin action as unique and freakish, but subsequent observations on several other severe cases indicated that it was by no means exceptional. Eventually we found in the literature records which showed the same erratic type of response to injected insulin as are shown in Table I. Because of the paucity and fragmentary character of pertinent quantitative material in the current literature, for satisfactory information we had to go back to the

TABLE III

SHOWING THE WIDE RANGE OF VARIATIONS IN THE RELATIONSHIP BETWEEN INSULIN DOSAGE AND THE AMOUNT OF CARBOHYDRATES UTILIZED (G/I RATIO)

Date	Diet (gm.)			Glucose (gm.)			Insulin (units)	G/I Ratio*
	P	F	CHO	Total Available*	Lost in Urine	Utilized*		
Allen's Case No. 54								
2/3/23	100	150	12	85	0	85	9	9.4
2/6/23	100	150	12	85	27	58	12	4.8
2/14/23	100	150	12	85	24	61	15	4.1
2/24/23	100	150	112	185	33	152	15	10.1
3/1/23	100	150	112	185	64	121	20	6.0
3/4/23	100	150	112	185	67	118	30	3.9
Allen's Case No. 85								
2/19/23	60	151	100	150	8	142	15	9.5
2/25/23	60	151	100	150	76	74	21	3.5
5/2/23	60	362	90	161	2	159	33	4.8
5/14/23	60	362	90	161	25	136	48	2.8
5/22/23	60	362	90	161	48	113	60	1.9
6/1/23	60	362	90	161	26	135	80	1.7
Allen's Case No. 328								
2/9/23	90	20	150	204	32	172	12	14.3
2/19/23	90	20	150	204	71	133	12	11.1
2/23/23	90	20	150	204	12	192	20	9.6
2/26/23	90	20	150	204	91	113	20	5.6
Allen's Case No. 1194								
1/2/23	100	100	25	93	22	71	16	4.4
1/4/23	100	100	25	93	25	68	16	4.2
1/22/23	100	100	25	93	42	51	50	1.0
1/23/23	100	100	25	93	32	61	50	1.2
2/17/23	90	171	200	270	56	214	50	4.3
2/19/23	90	171	200	270	18	252	50	5.0
Porges and Adlersberg's Case No. 73								
1/6/24	300 gm. meat			113	66	47	40	1.2
1/8/24	3 eggs, 30 gm. cheese (low fat)			113	60	53	60	0.9
1/13/24	80 gm. fat			113	39	74	80	0.9
1/27/24	100 gm. bread			113	4	109	112	1.0

* These values were computed from the data in the protocols of Allen and of Porges and Adlersberg.

TABLE IV

SHOWING THE CLOSE RELATIONSHIP BETWEEN THE RATIO R AND THE G/I RATIO

Date	Available Glucose in Diet (gm.)	Insulin (units)	R Ratio*	G/I Ratio†
Patient M. K. (from Table I)				
4/5/30	183	120	1.5	1.1
4/7/30	66	130	0.5	0.3
4/15/30	121	120	1.0	0.9
4/18/30	121	70	1.7	1.7
5/3/30	121	40	3.0	2.6
Allen's Case No. 54				
2/24/23	185	15	12.3	10.1
3/4/23	185	30	6.2	3.9
Allen's Case No. 85				
5/2/23	161	33	4.9	4.8
5/22/23	161	60	2.7	1.9
Allen's Case No. 328				
2/9/23	204	12	17.0	14.3
2/26/23	204	20	10.2	5.6
Allen's Case No. 1194				
1/2/23	93	16	5.8	4.4
1/22/23	93	50	1.9	1.0
2/17/23	270	50	5.4	4.3
2/19/23	270	50	5.4	5.0
Porges and Adlersberg's Case No. 74				
4/7/24	175	60	2.9	2.8
4/20/24	175	35	5.0	5.0
4/22/24	175	20	8.7	8.7

* $R = \frac{\text{Grams glucose available in diet}}{\text{Units insulin injected}}$.

† In order to simplify the table, the values of "G" (grams glucose utilized) were omitted.

early pioneering studies of Allen [*8*] and his associates [*9*] and of Porges and Adlersberg [*10*]. The problem which these workers set out to solve concerned the influence of the composition of diet on the insulin requirement of diabetic patients. It is noteworthy that although they used virtually identical experimental procedures, they arrived at quite different and rather conflicting conclusions. Porges and Adlersberg found that in diabetic subjects as well as in healthy persons, " . . . increase of the fat quota . . . given over extended periods of time, in most cases impairs the tolerance," which implies that high fat diets incur an increase in the insulin requirement. Allen, on the other hand, asserted that: "The most important factor governing the insulin requirement with any ordinary plan of diet is not the carbohydrate content but the total caloric content." This

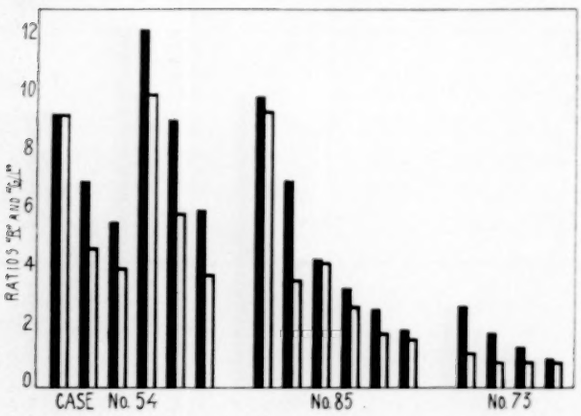


FIG. 1. Showing the interrelation between R and G/I ratios in three patients of Allen. Black bars represent the R ratio $\left(= \frac{\text{grams glucose available in diet}}{\text{units insulin injected}} \right)$. Blank bars represent the G/I ratio $\left(= \frac{\text{grams glucose utilized}}{\text{units insulin injected}} \right)$.

concept, as Allen actually applied it in his clinical practice, implied the view that diets containing relatively high fat rations do not affect the insulin requirement as long as fat replaces isocaloric amounts of carbohydrates and, to a limited extent, proteins.

On close inspection of the protocols of these authors it is revealed that Allen's conclusion was based on an erroneous interpretation of his own data. Since his coveted goal was the complete suppression of glycosuria by insulin treatment, he used, as did most other workers, the quantity of sugar *lost* in the urine as an index of insulin action instead of the amount *utilized* per unit of injected insulin. The capricious variations in the extent of glycosuria in his experiments actually showed no consistent relationship to changes in the composition of the diet; but when we computed from the protocols of Allen and of Porges and Adlersberg the amounts of carbohydrates that were utilized per unit of insulin, which is the appropriate yardstick for the appraisal of insulin action, an entirely different picture made its appearance.

Examples given in Table III will illuminate this picture. It may be seen that the G/I ratio shows in every instance essentially the same kind of variability that we had observed in our own studies. (Table I.) But on closer scrutiny of these figures a definite pattern emerges from the apparent chaos, revealing that the phenomenon which Allan observed in the depancreatized dog can be clearly recognized also in diabetic patients. This pattern presents the fact that the magnitude of the G/I ratio is largely dependent on the quantitative relationship between the amount of carbohydrates ingested and the amount of insulin injected, a relationship which can be expressed in the form of a ratio, R, which represents glucose available in the diet/insulin dosage. As may be seen in every case presented in Tables I and IV, and more clearly in the graph (Fig. 1), the G/I ratio increases when R increases, and shrinks when R decreases. The range of variability under the influence of a changing R ratio is very great; it may be noted in Table IV, for example, that Allen's Case No. 328 utilized as much as 14.3 gm. of carbohydrates per unit of insulin with a high R of 17.0, while at the other extreme our subject M. K. utilized only 0.3 gm. when R was reduced to 0.5. Insulin action evidently yields progressively diminishing returns as the R ratio decreases.

These facts help to explain the great divergencies in the "glucose equivalent of insulin" (G/I ratio) found by numerous authors, each presenting his figure as a standard stoichiometric equivalent. But they do not throw light on the mode of action of the R ratio; the results of studies addressed to this problem will be reported in a following paper.

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Exacerbation of Diabetes by Excess Insulin Action*

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IN a preceding paper [*1*] we presented evidence to show that the amount of carbohydrates utilized with the help of a given amount of injected insulin is variable, that it depends on the quantitative relationship between the amount of carbohydrates consumed by a subject and the amount of insulin injected. Denoting for simplified reference the relationship, *grams glucose available in diet/units insulin injected*, as the "R" ratio, we offered data culled from clinical protocols to show that as the numerical value of R increases or decreases, so does the value of the "G/I" ratio (grams glucose utilized/units insulin injected) increase or decrease. This fact was clearly demonstrated as early as 1923 by Allan [*2*] on depancreatized dogs, but was never correlated with clinical studies of insulin action.

The influence of the R ratio does not, however, fully account for the wide variations of the "glucose equivalent" of insulin. For one thing, there is no close proportionality between the changes in the R and G/I ratios; that is to say, while the two quantities change in the same direction, there is no close parallelism between them. This led to the assumption that some other causative factor or factors beside the R ratio are involved in the variations of the G/I ratio. This assumption found strong support in our observation under closely controlled conditions to the effect that the *blood sugar and glycosuria of diabetic patients often show wide fluctuations even when both the carbohydrate content of the diet and the insulin dosage are unchanged;* that is to say, the G/I ratio is subject to variations even when R is constant.

As we set out to find the cause of this anomaly, we found no guidance either in the clinical literature or in hospital records and discussions with eminent specialists in the field. We found no physiological concept for the appraisal of the insulin requirement of the diabetic patient except for the firmly rooted view that as a substitute for hypoinsulinism, the insulin dosage is to be adapted to the degree of the deficiency, which is reflected in the degree of hyperglycemia and glycosuria. But this rule of thumb fails to explain and to remedy the fluctuations in glycosuria and in the glycemic level, the variations in the amount of carbohydrates utilized while the insulin dosage remains the same. In this situation we had to launch our studies without a working hypothesis, with the negation of the existence of a generally valid glucose equivalent of insulin as the only firm point of departure. Hence we decided to proceed pragmatically: to accumulate facts under closely controlled conditions, then try to coordinate these facts.

PLAN AND METHODS OF STUDY

Subjects. Having decided on a direct approach to the problem, we chose as subjects diabetic patients whose hospital records showed ample evidence of the unexplained vagaries of insulin action. The first subjects were three young men who for a number of years had been treated in our hospital and clinic with large doses of insulin and were classified by the medical staff as the most severe, unmanageable diabetics on the roster of our clinic. They were invalids in the sense that they were unable to engage regularly in any kind of work and thus could spend any length of time under observation in a hospital ward, without economic sacrifice on their part.

Diet. In choosing a dietary regimen for our subjects we were guided by two well established facts. The first was the role of the ratio R, which furnished the information that much of the injected insulin is virtually wasted when given with diets containing small amounts of carbohydrates; and the waste is increased when, in addition, the proteins, as a potential source of glucose, are also restricted. In order to establish a high R ratio, we turned to diets containing liberal carbohydrate and adequate protein rations.

The second pertinent fact, well established by sev-

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eral authors [*3,4*], was that high fat-low carbohydrate diets cause pronounced deterioration of the glucose tolerance of healthy persons. We took it for granted that this rule is equally operative in diabetic subjects, and observations by Porges and Adlersberg on diabetic patients reinforced the validity of this concept.

It appeared imperative, then, to discard the high fat-low carbohydrate regimens, often coupled with restricted protein rations, which at that time (1935) were employed in all institutions in our own neighborhood as well as by the majority of clinicians everywhere. We used fats in the main to round out the individual caloric requirement; hence the fat ration was kept as low as possible for overweight persons, but was less restricted in patients in whom weight reduction was not necessary. While this regimen was primarily dictated by due regard to physiological laws, a practical aspect of it can hardly be overestimated: it made it possible to devise balanced menus scarcely different from those of healthy persons, a change that was greatly appreciated by diabetic patients who for years had lived on abnormal, unbalanced fare.

Evaluation of Insulin Action. While Porges and Adlersberg, as well as Allen and other workers, focused their attention on the amount of glucose excreted in the urine, we turned our attention from the negative to the positive aspect of the problem: we placed the primary emphasis on the amount of carbohydrates utilized by the patient, rather than on the amount lost in the urine. In the absence of any valid justification for the belief that glycosuria *per se* is to be dreaded, and convinced, furthermore, that efforts at immediate and lasting suppression of glycosuria in patients with severe diabetes, treated with substantial doses of insulin, were doomed to failure, we did not strive for immediate elimination of glycosuria but decided to tolerate relatively moderate degrees of it as long as the patient utilized an adequate quota of the carbohydrates consumed and did not show signs of adverse consequences.

Supporting Laboratory Tests. These consisted mainly of extensive *quantitative* determinations of urine sugar,* which were recorded in balance sheets, with the amount of carbohydrates ingested entered on the credit side, the amount lost in the urine on the debit side. The urine was collected in fractions from meal to meal, often also at brief intermediate intervals. Quantitative analysis of such fractions was expected to yield more detailed information about the extent as well as the chronologic course of glucose utilization, and hence of insulin action, than random determinations of fasting and postprandial blood sugar. At any rate, drawing of blood samples several times a day was impracticable in observations planned to extend over weeks and possibly months, while urines in any number of fractions could be collected without discomfort to the patient.

* In the course of the following years, as our studies embraced an ever increasing number of subjects, our laboratory had to run 200 to 300 quantitative urine sugar determinations daily. This would have required the full-time work of several technicians, using the Shaffer-Somogyi method for analysis, which was employed during the first year of the studies here reported. To overcome this handicap, we eventually devised a method for urine sugar determinations [*5*] which is as simple and rapid in execution as Benedict's qualitative test, so that one person can comfortably run 200 to 300 quantitative determinations a day. This method has completely replaced qualitative testing in our laboratory and in a number of other institutions.

OBSERVATIONS ON UNMANAGEABLE PATIENTS

Subject No. 1 was M. K., a thirty-six year old man; he was eminently qualified for our studies, owing to the informative hospital records covering the course of his diabetes over the preceding eight years. Here are a few salient points from his hospital records.

His diabetes was diagnosed by his family physician in 1927, when he was twenty-eight years old. The symptoms were loss of appetite, polyuria, polydipsia and weakness. A restricted carbohydrate and high fat diet failed to help, hence insulin was prescribed. This arrested loss of weight but because of distressing hypoglycemic reactions the patient soon stopped taking insulin. In November 1928, following an acute upper respiratory infection, his condition, acidosis with vomiting, required hospitalization. Attempts to keep him aglycosuric were unsuccessful and he was discharged from the hospital with instructions to take 70 units of insulin, half of it before breakfast, the other half before supper. His diet was 50 P, 120 F, 100 CHO. He was placed in the care of the outpatient division where his insulin dosage was juggled and increased in the customary manner in an effort to suppress glycosuria, but again without success. The patient had frequent and severe hypoglycemic reactions which, he later admitted, induced him occasionally to omit insulin injections; he suffered, furthermore, intense pangs of hunger which forced him to transgress in the dietary rules. Thus demoralized, he skipped most of his scheduled visits to the clinic.

On March 30, 1930, he was brought to the hospital in semi-conscious condition, dehydrated, vomiting, and with pronounced Kussmaul breathing. The comatose condition yielded only after injection of 385 units of insulin and subsequent intravenous administration of glucose. Then followed efforts—as frustrating as in the past—to render the patient aglycosuric, by

TABLE 1

EXCERPTS FROM LABORATORY RECORDS OF SUBJECT NO. 1 (M. K.), AN "UNMANAGEABLE" DIABETIC, SHOWING BEGINNINGS OF IMPROVEMENT

Date	Diet			Total Glucose Available (gm.)	Urine Sugar (gm.)				Glucose Utilized (gm.)	Insulin (units*)
	P (gm.)	F (gm.)	CHO (gm.)		B-L	L-S	S-B	Total		
9/26/35	120	150	100	185	1.8	0.4	22	24	161	30-0-30
10/4/35	70	30	250	294	13	7	13	33	261	25-10-25
10/7/35	70	30	250	294	28	0.8	25	54	240	25-10-25
10/8/35	70	30	250	294	29	18	25	72	222	25-10-25
10/9/35	70	30	250	294	13	1	6	20	274	25-10-25
10/10/35	70	30	300	344	34	30	25	89	255	25-10-25
10/11/35	70	30	300	344	32	21	13	66	278	25-10-25
12/28/35	70	60	275	322	24	27	43	94	228	25-10-25
12/29/35	70	60	275	322	16	4	2	22	300	25-10-25
12/30/35	70	60	275	322	16	36	29	81	241	25-10-25
12/31/35	70	60	275	322	16	14	47	77	245	25-10-25
1/17/36	70	60	275	322	15	34	5	54	268	25-5-30
1/18/36	70	60	275	322	3	6	3	12	310	25-5-30
1/19/36	70	60	275	322	14	4	14	32	290	25-5-30
1/20/36	70	60	275	322	8	3	27	38	284	25-5-30
1/21/36	70	60	275	322	7	8	4	19	303	25-5-30
1/22/36	70	60	275	322	14	26	28	68	254	25-5-30
2/23/36	70	60	275	322	9	13	34	56	266	25-5-25
2/24/36	70	60	275	322	7	18	9	34	288	25-5-25
2/25/36	70	60	275	322	0.3	10	6	16	306	25-5-25
2/26/36	70	60	275	322	16	28	5	49	273	25-5-25
2/27/36	70	60	275	322	2	16	29	47	275	25-5-25
2/28/36	70	60	275	322	11	3	44	58	264	25-5-25

* The three figures represent the three daily doses, injected before the meals.

juggling the insulin dosage between 60 and 180 units a day. Samplings from the erratic results of this procedure furnished the material for Table 1 in the preceding paper. After four weeks the patient was discharged on a diet of 50 P, 120 F, 100 CHO, and 60 units of insulin. Again in the care of the outpatient division, his insulin dosage was varied between 90 and 110 units per day, with the diet unchanged. During the next five years he was frequently hospitalized, on some occasions with severe acidosis, on others unconscious in hypoglycemic shock. He perennially commuted between home and hospital. During the next years "downward progress" continued to such an extent that by 1934 the patient was unable to work; conspicuous among his abnormalities was an enormous hepatomegaly, causing his abdomen to protrude while his body weight was subnormal.

The problems that presented themselves during the numerous hospitalizations of this patient had engaged the attention and elicited the comments and speculations of all the leading members of the medical staff. But all their concerted efforts at an acceptable "regulation" had remained fruitless. The patient was classified as an "unmanageable" diabetic.

This was the status of M. K. when he was hospitalized for our studies on September 25, 1935. After leaving the hospital on March 17, 1936, he continued with us as an ambulatory patient, collecting his urines quantitatively day after day in as many fractions as we deemed desirable, and maintaining daily contact with us. Motivated by appreciation of the radical improvement in his condition, which allowed him to return to a practically normal way of life, he cooperated with us loyally and reliably, enabling us to carry on uninterrupted observations on him for almost three years (October 1935 to August 1938).

Only excerpts of our voluminous records can be presented here, and even these but in condensed form, to illustrate our procedures and

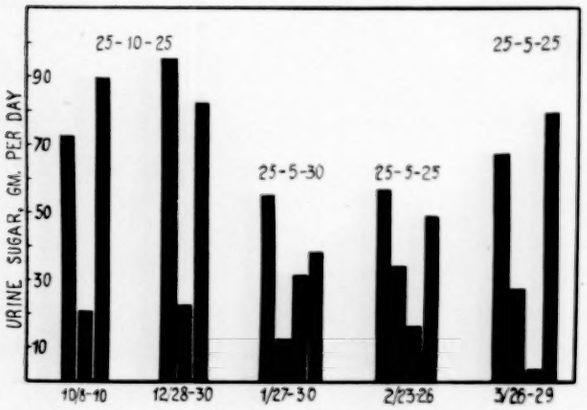

FIG. 1. Each black block represents urine sugar in grams per day. The ciphers above the blocks stand for the three doses of insulin injected thirty minutes before meals. The chart shows the cyclic recurrence of days with high glycosuria in the wake of days with low glycosuria. This pattern is characteristic of "unmanageable" diabetes.

results. It is to be noted, however, that these data are not offered as a pattern for practicable insulin therapy. Such a pattern has gradually taken shape in the course of many years of subsequent studies and will be described in another paper. Still, a survey of the path of our earliest empirical gropings, which finally led to a solution of our problem, may be of interest to other workers who have experienced and continue to encounter similar difficulties.

In Table 1 are presented samplings of the day-to-day laboratory records of the patient, accumulated during five months (October 1935 to February 1936) of continuous observation in the hospital. We included a sample of the six days in September during which the diet was the same as that prescribed by the outpatient division, but the insulin dosage was reduced to 60 units from 110, in order to give relief from distressing hypoglycemic reactions which attacked the patient at varying times of the day, sometimes shortly before the noon meal, on other days after 3 P.M., or between 10 P.M. and 1 A.M.—and not infrequently more than once in a single day. The G/I ratio on this diet was 161/60 = 2.7. When the diet was changed to yield 294 gm. available glucose, the G/I ratio increased substantially, but still showed variations between 3.7 and 4.6, although the ratio R was kept constant. This fact is brought into sharp focus by the day-to-day fluctuations of the glycosuria. As may be seen in Table 1, the amount of sugar in the urine during the October to December period ranged from 20 to 94 gm. per day. The picture is even more disquieting when, instead of lumping the urine sugar into twenty-four hour summaries, one inspects the great variations in corresponding fractions of consecutive days. For instance, on December 29 the spill was 4 gm. between lunch and supper, and 36 gm the next day; between supper and breakfast 2 and 29 gm. sugar were excreted, respectively on the same two days, and an alarming 47 gm. on December 31. Changes of this sort could not be accounted for by the influence of the ratio R.

Our efforts at remedying these unexplained vagaries remained futile until a close analysis of our balance sheets, accumulated in the course of three months, began to shed some light on the problem. When arranged in tabulated and graphic form, *a distinct pattern emerged* from the seemingly chaotic and perplexing mass. The pattern showed periodic fluctuations which seemed to recur in more or less regular waves or cycles, discernible in Table 1, and in sharper and unmistakable contours in the graph, Figure 1. A feature of this pattern which attracted our attention was the fact that high glycosuric tides tended to follow days when the sugar in one or two urine fractions was close to zero, even though the spill for the entire day may not have been conspicuously low. (We have reason to believe that during such low intervals unreported hypoglycemic states may have occurred.) The glycosuric tide that followed such days then gradually subsided in a few days, to yield to one or more low sugar days, only to mount again to another tidal wave. A fact that impressed us as very significant was that glycosuria flared up with especial violence in the wake of pronounced hypoglycemic reactions. A severe reaction, for instance, about 10 P.M. on October 9, was followed by a tidal wave of glycosuria next day; another such sequence occurred on December 29 and 30, when the glycosuria shot up to 81 gm. following a severe hypoglycemic shock during the night. The same phenomenon is shown in Figure 1, on March 28 and 29, 1936. The periodic ebb and tide pattern asserted itself so consistently that it suggested a *cause and effect relationship* between the two phases of the periodic waves: it appeared that *excessive glycosuria (hyperglycemia) is an aftermath of hypoglycemia.*

The validity of this assumption could be probed empirically by the application of a simple rule: avoid hypoglycemic reactions and see if, as a consequence, the occurrence of

hyperglycemic-glycosuric waves would be prevented. But it was easier to set up the rule than to follow it. When we tried to prevent hypoglycemic reactions by reducing the insulin dosage—we went as low as 40 units a day—the glycosuria became worse, but hypoglycemic episodes still occurred and the fluctuations between ebb and tide of the glycosuric waves became even greater than before. So we hurriedly returned to 60 units and instructed the patient to drink orange juice as soon as he perceived the slightest signs of oncoming hypoglycemia, in order to forestall its progress to a more serious stage. It took time before he was able to comply with this rule because, conditioned by past instructions, he was accustomed to take sugar only when "insulin reactions" manifested themselves in sweating and tremors. Now the patient was warned to recognize milder stages of hypoglycemia by the onset of a marked sensation of hunger, and to take fruit juice immediately.

This procedure bore favorable results. In January 1936, only a few hypoglycemic reactions of moderate degree were recorded, and, as may be seen in Table I, the range of fluctuations in glycosuria narrowed down appreciably. During February further progress in the same direction was in evidence, while the insulin dosage was lowered by 5 units. As another sign of improvement, ketonuria, which often appeared in various fractions of the urine, occurred but rarely after December and was completely eliminated by February. Simultaneously, identical results were obtained with two other unmanageable diabetic patients, so that we felt justified in postulating *a cause and effect relationship between hypoglycemia and the ensuing tidal waves of glycosuria*.

Along with the favorable changes that were reflected in the laboratory data, the patient showed spectacular clinical improvement: his huge liver receded to normal size, muscular weakness and fatigue disappeared, and he emerged from his demoralized, hopeless looking condition with the attitudes of a healthy, normal man. At this juncture we regarded the patient as rehabilitated from his near invalid state of an "unmanageable" diabetic, yet the still too high levels and considerable fluctuations of his glycosuria made it clear that our task was by no means finished. But because further prolongation of hospitalization seemed, mainly for psychological reasons, unsound, the patient was discharged on March 17, 1936, with the understanding that for a while he was to collect his urines quantitatively in any number of fractions we desired, measure the volume of each fraction, and come with samples of each daily to the hospital. He was instructed to report the slightest symptoms of hypoglycemia, as well as any other self-observations, and to receive advice and directives after the urine samples had been analyzed. His diet was set at 70 P, 225 CHO, 60 F, and his insulin dosage at 55 units, divided in doses of 25-5-25 units, as it was in the hospital.

Adaptation to home life incurred some difficulties which, in view of the limited intelligence of this patient, were not unexpected; but his sincere and devoted cooperation helped to compensate for this handicap. In Table II are recorded data showing changes in the patient's condition during the next eight months. For the sake of economy, we present only greatly condensed material, constructed from protocols containing daily fractional urine sugar deter-

TABLE II
SHOWING GLYCOSURIA, AVERAGES AND RANGE OF DAY-TO-DAY FLUCTUATIONS, DURING A PERIOD OF EIGHT MONTHS
(APRIL 1 TO DECEMBER 10, 1936)

Date	Urine Sugar (gm. per 24 hr.)		Remarks
	Average	Range of Fluctuations	
4/1/36–4/10/36	58	33– 85	Mar. 18–June 25
4/11/36–4/20/36	67	30–105	Insulin dosage, 55 units (25-5-25); diet, 70 P- 60 F- 225 CHO, yielding 272 gm. available glucose per day, of which, on an average, about 215 gm. were utilized
4/21/36–4/30/36	65	31–105	
5/1/36–5/10/36	64	37– 78	
5/11/36–5/20/36	54	12– 72	
5/21/36–5/30/36	54	21– 72	
5/31/36–6/9/36	49	21– 72	
6/10/36–6/20/36	54	34– 68	(June 22–July 17, hospitalized for studies)
7/20/36–7/29/36	29	18– 42	
7/30/36–8/8/36	18	2– 25	June 26–July 12
8/10/36–8/19/36	21	Trace– 35	Insulin dosage, 60 units (25-5-25-5*); diet, CHO raised to 275 gm., increasing available glucose to 322 gm., of which about 295 gm. were utilized
8/20/36–8/29/36	26	18– 35	
8/30/36–9/9/36	30	16– 40	
9/10/36–9/19/36	33	14– 54	
9/20/36–9/29/36	22	Trace– 59	
10/1/36–10/10/36	25	Trace– 41	
10/11/36–10/20/36	8	0– 37	July 13–Oct. 12
10/21/36–10/31/36	4	0– 42	Insulin dosage, 58 units (25-5-25-3*); diet, unchanged
11/1/36–11/10/36	27	18– 36	
11/11/36–11/17/36	12	2– 36	
11/21/36–11/30/36	10	0– 36	(July 18, discharged from hospital)
12/1/36–12/7/36	16	Trace– 39	From Oct. 13 on the 3 A.M. dose was dropped, thus the insulin dosage was 55 units (25-5-25); diet, unchanged

* At 3 A.M.

minations for the entire period. The figures stating the range of day-to-day fluctuations of the glycosuria and the averages, mostly for ten-day periods, of the amounts of sugar excreted give a fair picture of the changes in the patient's condition.

As may be noted in Table II, during the first month (April) at home the glycosuria was worse than in the hospital, both as regards the maximum levels (105 gm. in twenty-four hours) and the range of fluctuations. The main reason for the deterioration, it was ascertained, was that the patient frequently was remiss in the prevention of hypoglycemic reactions. Persuaded of the harmfulness of such neglect, he became more diligent and as a result, in May and June, the picture improved somewhat; but the much too wide day-to-day fluctuations of the glycosuria (as, for instance, a range of 12 to 72 gm. for the period of May 11 to 20) demanded further close studies. Convinced that increased glycosuria appears as an aftermath of hypoglycemic reactions, we began to suspect that the same effect is produced, to a lesser extent, by moderate degrees of hypoglycemia which cause no perceptible subjective symptoms. For a closer study of the problem the patient was again hospitalized on June 22, 1936.

At this stage of our observations blood sugar determinations would have been illuminating but we continued to rely on the analysis of fractional urine specimens because obtaining of blood samples at the right moment is often difficult, and to subject the patient to venipuncture around the clock, day after day, is hardly practicable. Collection of urine samples every few hours for several consecutive days, in addition to the data accumulated during the preceding months, seemed to throw some light on our problem. In the midst of considerable variations a general pattern emerged, showing a sharp drop in the urine sugar during the fourth hour after supper (nearly a postabsorptive state), followed by a sugar-free fraction between 10 P.M. and 2 A.M. This coincided with the interval during which, time and again, frank hypoglycemic reactions were observed. It seemed reasonably certain, therefore, that many more hypoglycemic states occurred during these aglycosuric intervals, but were not severe enough to arouse the patient from sleep or to attract the nurse's attention. After such sugar-free, or nearly sugar-free intervals, glycosuria made its appearance and rose steeply until 7 A.M., when the morning dose of insulin was injected. The glycosuric tide continued to assert itself for about two hours after breakfast, then it subsided abruptly, so that after 11 A.M. the urine became sugar-free, and a hypoglycemic tendency appeared either near noon, or a few hours following the noon meal. To smooth out this ebb-and-tide profile, on June 26 two measures were introduced. In the first place we gave the patient some fruit at 10 A.M., and one slice of bread and fruit at 10 P.M. (about four hours after supper), in order to forestall the precipitous fall of the blood sugar. The carbohydrate ration thereby was raised to 275 gm., from the previous 225 gm. per day. Secondly, we resorted to the injection of *5 units of insulin at 3* A.M. in order to check the glycosuric tide at its inception. This second change was not intended as a permanent part of treatment—it was but one of our empirical steps. Table II shows at a glance the immediate benefits of these measures: by the end of July the maximum, as well as the average levels of glycosuria had diminished and the range of fluctuations had narrowed down substantially.

The 3 A.M. dose of insulin soon displayed an unexpected effect: its action depressed the glycosuric tide with rapidly increasing efficiency, so that in a week the urine excreted between 3 and 7 A.M. became nearly or completely sugar-free. In another few days the patient reported clinical symptoms of hypoglycemia at about 6 A.M. This necessitated, on July 13, reduction of the 3 A.M. dose to 3 units. On July 18 the patient was sent home with instructions to follow the same regimen as in the hospital; he was again followed up on an ambulatory basis. As may be seen in Table II, improvement continued, so that during the first ten days of October nearly sugar-free days began to crop up. By this time the 3 units of insulin, injected at 3 A.M., began to induce hypoglycemic symptoms between 6 and 7 A.M.; in consequence, on October 13 this dose was abandoned. To our gratification, the glycosuric wave which had prompted the introduction of the 3 A.M. dose did not reappear.*

* At that time (1936) we were puzzled as well as gratified by the fact that the beneficial effect of the 3 A.M. insulin dose persisted after the injection had been discontinued, but were unable to understand it. In the course of our continued studies we observed manifestations of this phenomenon in several forms and variations and found an explanation for it. It has to do with *conditioned patterns* in the homeostasis of the glycemic level, a subject which will be treated in a separate paper.

TABLE III

SHOWING GROSS OVER-INSULINIZATION OF SUBJECT NO. 1 WHEN "REGULATED" WITH 55 UNITS INSULIN

Date	Urine Sugar (gm. per 24 hr.)		Insulin (units)	Remarks
	Average	Range of Fluctuations		
On Protamine-Zinc Insulin				
12/8/36–12/14/36	16	3–29	25-0-25	12/9/36, severe reaction with coma at 8 P.M.
12/18/36–12/21/36	20	0–49	25-0-15	12/11/36, severe reaction with coma at 3 A.M.
12/22/36–12/31/36	15	0–25	20-0-15	12/20/36, reaction at 1 P.M.
1/5/37–1/14/37	28	3–69	20-0-6	12/31/36, reaction between 5 and 6 A.M.
3/23/37–3/31/37	32	5–77	10-0-6	1/6/37, reaction at noon and 3 P.M. (on 26 units a day).
4/21/37–4/30/37	23	Trace–50	10-0-10	.....
				Blood sugars:
5/21/37–5/30/37	30	Trace–61	.....	5/15/37, fasting, 264 mg. %
6/21/37–7/4/37	25	Trace–51	.....	5/20/37, 10:30 A.M., 73 mg. %
7/19/37–8/1/37	28	9–43	.....	.....
8/6/37–8/13/37	29	Trace–54	8-0-12	.....
On Crystalline Insulin				
8/14/37–8/27/37	28	4–49	8-0-12	The fourth dose of 5 units was started on 1/29/38 and was given at 11 P.M., together with a slice of bread and an apple; as a consequence the morning dose became immediately excessive and had to be stepwise reduced from 8 units to 5 units
9/11/37–9/26/37	48	15–71	.....	
10/11/37–10/24/37	34	5–50	.....	
11/8/37–11/21/37	35	10–52	.....	
2/20/38–2/28/38	17	7–26	5-0-6-5	
3/1/38–3/10/38	23	12–33	.....	
4/10/38–4/19/38	15	7–29	.....	
5/11/38–5/23/38	19	6–37	.....	
6/13/38–6/22/38	17	5–30	.....	
7/14/38–7/23/38	19	9–28	.....	

Comparison of the laboratory data of October to December, 1936 (Table II), with those in Table I shows the substantial progress that had taken place in the patient's condition in the course of one year. Glycosuria was greatly reduced and there were many sugar-free days; clinically, the patient was no longer a demoralized, "unmanageable" diabetic. But welcome as this improvement was, the laboratory data still posed questions which could not be simply shrugged off: why, we had to ask, were sugar-free days still followed by days with 35 to 40 gm. urine sugar, although no hypoglycemic symptoms were observed? We still suspected, however, that mild degrees of hypoglycemia, which eluded observation, may have been responsible for this anomaly. It was at this time that protamine-zinc insulin (PZI) became available. We resorted to the widely hailed new preparation mainly for the reason that its action being more evenly spread and less aggressive than that of plain insulin, its use would at least mitigate, if not obviate, the wide oscillations of the glycemic level which may harbor episodes of mild hypoglycemia. It was also expected that the prolonged action of PZI would make it possible to eliminate the 5 unit dose injected before lunch.

As the insulin dosage was switched from 25-5-25 units of plain insulin to 25-0-25 PZI (the diet remained unchanged), we entered an uncharted territory and found it necessary to hospitalize the patient for close observation. This precaution proved to be well justified, for from the very outset we had our share of surprises. One fact that ran contrary to our expectation was that instead of a more even response to insulin action the range of day-to-day fluctuations in glycosuria became worse than it had been with plain insulin, and hypoglycemic

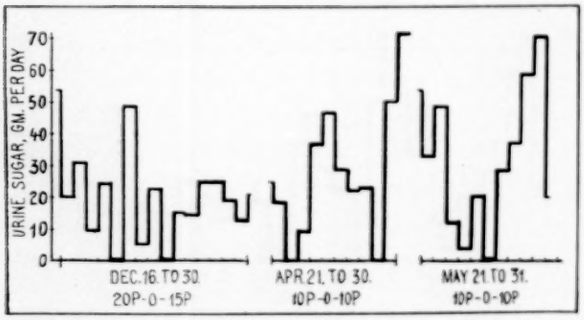

FIG. 2. This graph shows that protamine-zinc insulin, as used in this experiment, failed to remedy the wave-like alternation between sugar-free (or low glycosuric) days and days of excessive glycosuria. Frequent hypoglycemic reactions, which occurred with 20 units PZI per day, invariably entailed excessive glycosurias in the manner characteristic of unstable diabetes.

reactions, which we succeeded in eliminating while using plain insulin, returned with a vengeance after the switch to PZI. These observations are recorded in the upper half of Table III, of necessity again only as extremely condensed excerpts from our protocols, which comprise complete day-to-day sugar determinations in fractionally collected urines.

As may be seen in Table III, on December 9, the second day on the new regimen, at 8 P.M., only three hours after supper, the patient had a hypoglycemic reaction which was so severe that it caused unconsciousness before it was noticed. He was given 90 gm. glucose, 25 gm. of it intravenously, before he recovered. After several reactions of less severe degrees, on December 11 at 3 A.M. he was once more found unconscious and had to be revived with intravenous glucose. Reduction of the insulin dosage seemed mandatory and was carried out as the fractional urine sugars and hypoglycemic reactions dictated. Still, on December 20, when the dosage was cut to 35 units per day, a severe reaction occurred at 1 P.M., directly after the noon meal, and on December 31 a similar reaction was intercepted between 5 and 6 A.M. Further cuts in the dosage were made, yet on January 6, when the insulin dosage was as low as 26 units a day, two hypoglycemic reactions took place, one at noon, the second at 3 P.M. In consequence, we stepwise reduced the dosage to 16 units a day. During the period of March 23 to 31, this was sufficient to keep the glycosuria as low as 5 gm. on some days (Table III); but such days were inexorably followed by intolerable glycosuric tides, rising above 70 gm. Subsequently, with 20 and 22 units of PZI per day the fluctuations persisted, nearly sugar-free days alternating with days of over 50 gm. urine sugar. Hypoglycemic episodes still occurred; they crept up insidiously, so that the patient was unable to perceive their approach and prevent their progress in time. Further cut of the insulin dosage was contraindicated by the fact, clearly discernible in the graph (Fig. 2), that the glycosuric tide, which was entailed by sugar-free days, increased both in its vertical and horizontal dimensions when the insulin dosage was reduced.

In retrospect, we are aware that unfamiliarity with the nature of PZI action played a part in our difficulties. Nevertheless we derived from this experiment the invaluable information that we grossly overinsulinized the patient by treating him with 55 units of plain insulin in the preceeding period. He was physically and mentally well enough on that dosage to be regarded as a "well controlled" diabetic, certainly better beyond comparison than he was when getting 90 to 110 units before becoming a subject of our studies. He had been rehabilitated from an invalid state, and we had no basis or precedent to indicate that he could get along with appreciably less insulin than 55 units. We changed to PZI in the hope that this change might narrow down the day-to-day fluctuations of his moderate glycosuria. While this endeavor was unsuccessful, it brought the revelation that *the patient required no more than about 20 units of insulin for the utilization of approximately 300 gm. glucose, representing a G/I ratio of 15* (serendipity!)

Thus, when in our predicament we abandoned PZI and reverted to the use of plain insulin we did not go back to 55 units, but simply replaced the 20 units of PZI last used with the same amount of crystalline insulin. The results of this change are presented in the lower part of Table III. As may be seen from the condensed data, the average level of glycosuria remained about the same as with PZI, but the range of fluctuations became narrower, although it was still far too great to give us comfort. These fluctuations—as fractional urine sugar determinations revealed—were caused by the excessive action of the 12 units of insulin given before supper, which time and again precipitated mild hypoglycemic symptoms around 10 or 11 P.M. For this reason we divided the evening dose into two fractions, giving 6 units before supper and 5 units at 11 P.M. Together with this last dose the patient received a slice of bread and an apple of about 4 ounces.

This step once again brought into sharp focus *the harmful effect of hypoglycemia:* when its occurrence during the fifth or sixth hour after supper was prevented, not only was the glycosuria during the ensuing night hours eliminated, but the beneficial effect was extended to the next morning. This was reflected in the fact that the 8 units of insulin injected before breakfast became excessive, so that the subject now needed orange juice at 10:30 A.M. in order to stave off hypoglycemic symptoms which appeared about 11 A.M. In consequence the morning dosage was cut in 1 unit gradients to 5 units. After these adjustments (end of January 1938), the patient was getting along very well on 16 units of plain insulin per day. As may be seen in the lower half of Table III, in the course of six consecutive months, on a diet supplying better than 300 gm. of glucose per day, the average daily spill was a little below 20 gm. and the range of day-to-day fluctuations narrowed down considerably. The patient enjoyed good health and was able to pursue a normal way of life.

It is proper to enquire at this point whether this man was a unique, exceptional case, or is it permissible to conclude that his unmanageable state, from which he emerged as a mild diabetic, was the product of over-insulinization (insulin intoxication)? We can offer to this question two answers. In the first place, in the latter part of 1938 we subjected this patient to an experiment which proved that he promptly reverted to an unmanageable state as soon as his insulin dosage was increased above his actual requirement; this experiment will be described in the next section of this paper. Secondly, during the period of time encompassed by the foregoing studies we applied the same procedure and treatment to a number of other unmanageable diabetic patients who, without exception, responded in the same manner as this subject.

Subject No. 2. H. C., a well developed twenty-three year old man, was admitted to the hospital for our studies on September 22, 1935. A rather acute onset of polyuria, polydipsia, glycosuria, ketonuria and rapid loss of weight led to the diagnosis of diabetes three years earlier. His physician, a reputable internist, gave him a diet of 65 P, 70 F, 100 CHO, with 45 units of insulin per day. On this regimen glycosuria was controlled only for a short period and as a consequence the insulin dosage was gradually increased to 90 units. Owing to the economic depression, a year later our subject became a staff and clinic patient. In January 1935 he was hospitalized in a state of severe acidosis and after one week of regulation was discharged on a diet of 70 P, 135 F, 80 CHO, with 95 units of insulin. On the day of his discharge his urine was sugar-free, but this was merely incidental, for as an ambulatory patient he was soon classified as an unmanageable case.

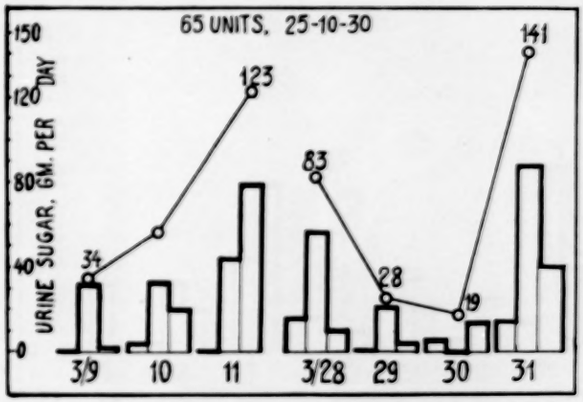

FIG. 3. Showing the cyclic ebb and tide pattern in the glycosuria of Subject No. 2, an infallible evidence of over-insulinization. Each heavily contoured block represents the urine sugar for one twenty-four hour period; the lighter lines divide the blocks into three sections, representing the urine sugar from breakfast to lunch, lunch to supper, and supper to breakfast. The circles show the total urine sugar per day.

With these antecedents he entered the hospital as a subject for our studies. This young man was virtually an invalid, unable to fill any job for any length of time. He was quite demoralized by all too frequent, severe insulin shocks, which entailed periods of weakness and profound depression. As he eventually initmated, time and again he considered suicide.

He was immediately given a low fat, liberal carbohydrate diet and was given 105 units of insulin to combat his acidosis. We followed much the same empirical procedure as with Subject No. 1, and for several months encountered similar difficulties. There were, of course, individual differences; thus, we had to combat higher glycosuric tides and more severe episodes of ketosis, which on two occasions advanced to a state of pronounced acidosis. In March 1936, the sixth month of rather frustrating efforts to stabilize his condition, the patient was still receiving as much as 65 units of insulin per day (distributed as 25-10-30). As may be seen in the graph (Fig. 3), the glycosuria still exhibited the extreme waves of ebb and tide: on March 30, for instance, he spilled 19 gm. sugar, on the next

TABLE IV

SHOWING THE PROGRESS OF SUBJECT NO. 2 (H. C.) FROM THE STATE OF SEVERE DIABETES TO A MILD STATE OF THE DISEASE, EFFECTED BY ELIMINATION OF EXCESS INSULIN ACTION

Date	Urine Sugar (gm.)				Insulin (units)	
	B-L	L-S	S-B	Total	Total	Distribution
4/15/36	14	62	0	76	61	25-6-30
4/16/36	10	0	76	86		
4/17/36	8	10	0	18		
4/18/36	2	47	10	59		
11/4/36	4	0	0	4	45	20-10-15
11/5/36	0	0	0	0		
11/7/36	0	0	20	20		
11/14/36	14	0	0	14	39	18-6-15 (since 11/11/36)
11/15/36	12	9	0	21		
11/16/36	0	0	0	0		
11/21/36	29	16	8	53	36	15-6-15 (since 11/19/36)
11/24/36	4	0	27	31		
2/11/37	0	0	0	0	23	10-5-8
2/12/37	1	18	19	38		
2/13/37	18	8	31	57	20	12-0-8
2/14/37	13	0	6	19		
2/15/37	2	2	16	20		
3/3/37	4	18	11	33	18	10-0-8
3/4/37	7	10	0	17		
3/10/37	4	10	0	14		
3/11/37	5	1	1	7		
3/17/37	0	0	0	0		
4/5/37–4/8/37	0	0	0	0		

day 141 gm. But ketonuria was finally subdued, severe hypoglycemic reactions were staved off, at least in the patient's waking hours, and subjectively he felt definitely better than prior to his hospitalization. He insisted on resuming his work as a sales clerk and, not without misgivings, we discharged him from the hospital, to be followed daily on an ambulatory basis.

In the second week out of the hospital the patient reported that he had begun to suffer hypoglycemic reactions shortly after midnight, although he ate a slice of bread and an apple at 10 P.M. These reactions were followed by glycosuric tides, and for this reason the 30 unit dose of insulin before supper was cut to 20, then to 15 units. But then the glycosuria during the night became intolerably high, and the tide did not subside until about 10 A.M. As in Subject No. 1, we combatted this anomaly by giving a temporary injection at 3 A.M., starting with 10 units, and gradually lowering and finally eliminating it (see footnote on Subject No. 1).

By June 1936 the patient was on 20-10-15, a total of 45 units of insulin per day, with about 350 gm. of available glucose in his meals. Feeling well and happy, he unilaterally decided that further regulation and contact with us had become unnecessary. We were unable to persuade him that the day-to-day fluctuations of his glycosuria indicated that his insulin dosage was undoubtedly still excessive.

In October 1936, however, the patient voluntarily returned. He was alarmed by frequent and increasingly severe hypoglycemic reactions, which occurred near noontime, sometimes while eating his lunch, and also at about 4 or 5 P.M. Reactions in the late evening also appeared, although but infrequently; the bread and apple he consumed at 10 P.M. averted such episodes, and they occurred only when he omitted this extra food. Since June his sensitivity to insulin action had evidently improved and, being a rather intelligent person, he realized that the course of adjustment of his insulin dosage had been prematurely interrupted. The patient now offered to resume the collection of fractional urine specimens from day to day, and he conscientiously did so from November 4, 1936 to March 15, 1937. The data given in Table IV, which represent excerpts from our records for this period, show clearly enough how the results of fractional urine sugar determinations served to guide our course in reducing the insulin dosage, until it shrank to 18 units a day, distributed as 10-0-8 units. At this point the patient soon became consistently aglycosuric and daily testing of the urines became superfluous. In April 1937 tests on four consecutive days were negative, and further tests were restricted to one or two twenty-four-hour summaries per week.

We were reasonably certain that the insulin dosage could and should be further reduced below 18 units, but the patient was happy with his status and asserted that he had learned enough to be able to forestall even the slightest degree of hypoglycemia. He has never been hospitalized since discharge from the hospital in March 1936, and after May 1937 he withdrew from any further laboratory studies beyond bringing to us from time to time, for about another year, samples of twenty-four-hour urine summaries; these were consistently sugar-free.

Currently he is still using 18 units of insulin per day, is a prosperous merchant and enjoys perfect health. Two interesting episodes, attesting to his good physical condition, are worthy of note. In 1942 the military draft board classified him as "1A," and in the busy army induction center, after physical examination, he was about to be branded a malingerer when a glu-

cose tolerance test, performed at the patient's request, finally convinced the doctors that the man had diabetes. In 1955—twenty years after becoming a subject in our studies—when he applied for life insurance, in view of his diabetes he was subjected to thorough medical examination, especially for retinopathy and vascular degeneration. His condition was found to be perfectly normal in every respect.

Subject No. 3. A. S., a twenty-seven year old man, was the third in the first group of unmanageable diabetics hospitalized for our studies in October 1935. His diabetes was diagnosed in 1931 and he was immediately placed on a regimen of low carbohydrate diet and insulin (information as to dosage not available). In 1932 he was taken to a university hospital in a state of diabetic coma and was discharged on an insulin dosage of 75 units per day. In September 1933 he was admitted to our hospital, once more in a state of coma. When discharged he was placed on a diet of 80 P, 150 F, 80 CHO, with 65 units of insulin daily. One week later he was back in the hospital with severe acidosis and complaints of frequent, severe hypoglycemic reactions. He was discharged on the same diet and 60 units of insulin and referred for weekly checks to the outpatient division. Spot tests of the urine showed occasional aglycosuria, but more often massive glycosuria and ketonuria.

This went on for a year and a half; then in January 1935 the patient, complaining of severe and frequent hypoglycemic reactions overtaxing his endurance, was readmitted to the hospital. He showed marked glycosuria and ketonuria. To combat this condition, his insulin dosage was raised to 90 units, distributed as 40-20-30 units. He left the hospital on this regimen and a diet of 70 P, 135 F, 80 CHO. Hypoglycemic reactions, along with glycosuria and ketosis, continued to plague him. He was unable to keep any steady job and was depressed and worried. He also was deeply disturbed by his inability to stick to the prescribed 1,800 calorie diet, and asserted that under the urgency of intense hunger and hypoglycemic reactions he was unable to avoid overeating. His body weight was 20 pounds above normal.

This was the patient's condition when he was hospitalized for our studies on October 7, 1935. During the first two days, while he was kept on his old regimen, i.e., 90 units of insulin per day, with carbohydrates restricted to 80 gm., he excreted 30 and 50 gm. of sugar, the bulk of it during the night, and showed pronounced ketonuria in every fraction of the urine. Then we changed the diet and manipulated the insulin dosage, following essentially the same procedure as with Subjects No. 1 and No. 2.

TABLE V
SHOWING PROGRESSIVE IMPROVEMENT IN THE CONDITION OF SUBJECT NO. 3 (A. S.)

Date	Urine Sugar (gm. per 24 hr.)		Insulin (units)	
	Average	Range of Fluctuations	24 hr.	Distribution
1/1/36–1/10/36	66	57–100	58	20-3-35
2/11/36–2/20/36	37	0– 65	55	25-0-30
4/11/36–4/20/36	59	0– 95		
7/21/36–7/30/36	56	0–101	50	20-0-30
8/21/36–8/31/36	31	0– 70		
9/23/30–9/30/36	10	0– 22	45	20-0-20-5*
11/11/36–11/20/36	26	3– 48	39	16-0-20-3
11/21/36–11/28/36	16	0– 44	36	16-0-20

* At 4 A.M., beginning 9/20/36.

In this instance ketonuria was more easily overcome, and when the patient insisted that he felt capable of returning to work as a truck driver, he was discharged on December 16 and thereafter treated as an ambulatory patient. He left the hospital with instructions to follow a diet of 100 P, 300 CHO, 60 F, which included intermediate snacks for staving off hypoglycemic reactions. The insulin dosage was 58 units per day, distributed as 20-3-35. The further steps were similar to those applied in the instance of Subject No. 2. A brief summary of the procedure, given in Table v, shows that by the end of November 1936 the dosage was reduced to 36 units, and glycosuria fluctuated from zero to 44 gm. per day, as against zero to 100 gm. earlier in the year, i.e., before the introduction of an insulin dose at 3 A.M. However, these fluctuations were still indicative of excessive insulin action and of the need for further downward adjustments of the dosage. The patient, however, harking back to his condition of a year ago, was happy and satisfied with his condition. He was working steadily and found that his occupation made it impossible for him to submit to further studies.

Five years later (1942) he returned to us. During that period he had had no medical care, as he felt that he was able to get along without it. In the meantime he had gradually increased his insulin dosage to 70 units, motivated by stray information that a diabetic patient could eat

TABLE VI

TRANSITION IN SUBJECT NO. 4 (F. C.) FROM SEVERE, UNSTABLE STATE TO MILD DIABETES

Date	Urine Sugar (gm.)			Insulin (units)		Remarks
	B-S	S-B	24 hr.	24 hr.	Distribution	
1/27/36	..	..	148	30	15-0-15	In care of clinic; diet, 70 P, 50 F, 250 CHO; remained an "unmanageable case"
1/28/36	..	..	67			
1/29/36	..	..	265			
1/30/36	..	..	110			
2/1/36–2/9/36	..	..	66–123			
2/11/36	22	98	120	50	25-0-25	
2/20/36	39	73	112			2/20 severe hypoglycemic reaction at midnight; 2/23 hypoglycemic reaction at 7 A.M.
2/21/36	41	81	122			
2/22/36	49	43	92	50	10-0-25-15*	
2/23/36	22	24	46			
2/24/36	35	13	48	50	20-0-20-10*	
2/25/36	42	16	58			
2/26/36	27	7	34			
2/27/36	23	10	33			
2/28/36	3	2	5			Severe hypoglycemic reactions recorded: 3/3, 1 A.M.; 3/6, midnight; 3/8, 11:30 P.M.; 3/10, 10:30 P.M.; 3/19 and 3/20, between 4 and 5 P.M.
3/7/36	..	..	38	45	20-0-15-10*	
3/8/36	..	..	34			
3/9/36–3/12/36	..	..	11–15	40	20-0-10-10*	
3/14/36–3/24/36	..	..	7–30	35	15-0-10-10*	
5/6/36	..	..	45	32	15-0-10-7*	
5/13/36, 5/15/36, 5/20/36	..	..	0			
5/22/36	..	..	0	23	15-0-8	
5/23/36–5/31/36	..	..	0			
6/5/36–6/11/36	..	..	0	20	10-0-10	

* Injected at 4 A.M.

more and more if only his insulin dosage were increased along with the added food. As a result he was grossly overweight and felt chronically fatigued and weak. His glycosuria was excessive and he had ketonuria. He was well on his way to the same condition which before 1936 had twice thrown him into diabetic coma and had made him a semi-invalid. The late Dr. H. V. Goldwasser took him into his care and, on the basis of what we had learned by 1936 and afterwards, gradually improved his condition, ending with an insulin dosage of 26 units per day. The patient's diabetes remained under fair control, but four years later he died of pulmonary tuberculosis.

Subject No. 4. Mr. F. C., a twenty-six year old man, was known to have had diabetes for five years when he came under our observation at the end of February 1936. In 1931 he was in a university hospital in diabetic coma and, after several weeks of treatment, was discharged with instructions to follow a diet of 60 P, 125 F, 100 CHO, with 50 units of insulin per day. In January 1933 the economic depression brought him to the free clinic of the Jewish Hospital, where his diet was changed to 70 P, 140 F, 80 CHO, and, with regard to his frequent hypoglycemic attacks, his insulin dosage was lowered to 40 units; as hypoglycemic reactions were not mitigated by this step, the CHO ration was soon raised to 120 gm. He still remained very unstable, suffering from frequent hypoglycemic reactions while variable degrees, often very heavy, of glycosuria and ketonuria persisted.

Perturbed by the lack of improvement in his condition, early in 1935 the patient returned to private medical care. His physician gradually raised the insulin dosage to 75 units per day, without changing the diet; but his condition showed further deterioration and this induced him, in January 1936, to come back to our clinic. There the physician, who was aware of our studies on the three subjects just described, made a radical change in the patient's regimen. In an endeavor to avoid the distressing hypoglycemic reactions—and informed that these entail

glycosuric tides—he radically lowered the insulin dosage to 30 units (15-0-15) per day from the previous 75 units and at the same time changed the diet to 70 P, 250 CHO, 50 F. The result was quite unsatisfactory. As may be seen in Table VI, on one day (January 28) he spilled 67 gm. of sugar, not too disturbing in relation to about 300 gm. available glucose ingested, but on the next day glycosuria shot up to 265 gm. Hypoglycemic reactions still occurred and ketonuria was persistent. By February, on the same regimen, ketonuria subsided and the glycosuria was somewhat tamed (still 66 to 123 gm. per day); hypoglycemic reactions became less frequent and less severe, mainly because the patient was instructed to eat some fruit at the earliest signs of the approach of a reaction.

At this juncture, on February 11, 1936, the patient was hospitalized for our studies. Making rapid progress in two weeks, he insisted on going back to work (he was a baker), as he subjectively felt able to do so, and—not without misgivings—we agreed to continue his treatment on an ambulatory basis. While he was in the hospital fractional urine samples disclosed the fact that by far the greater part of the sugar was excreted during the night, as for instance on February 11 (Table VI); more detailed fractioning showed, moreover, that the bulk of the nightly spill occurred after 4 A.M., while the fraction between supper and midnight contained relatively little sugar and the midnight to 4 A.M. fraction showed only negligible amounts or none at all. It appeared a logical step to attempt to stem the glycosuric tide by meeting it at its inception with an injection of insulin at 4 A.M. On February 22, we chose 15 units of insulin to serve this purpose. This dose proved to be too much and was lowered to 10 units, with 100 cc. orange juice, soon 150 cc., given at the same time. In fairly rapid succession the dosages had to be gradually cut at the other two spots also, and by May 13 the patient was aglycosuric and remained so consistently on two 10 unit doses a day. The data in Table VI show the progressive improvement clearly enough to make added comment superfluous.

On the basis of our records, it can be stated that this twenty-six year old unmanageable diabetic who, shortly before coming under our observation, had been treated with 75 units of insulin per day, six months later was consistently aglycosuric on 20 units per day, with a liberal balanced diet, and was able to live and work like any healthy man. Since the end of 1936 he has not returned to us, or—to our knowledge—to any hospital or outpatient clinic.

Subject No. 5. At the end of October 1935, when the improvement in the condition of our first group of unmanageable study cases (Subjects No. 1, No. 2 and No. 3) had come to the attention of one of the senior members of the medical staff, he requested our collaboration in the treatment of one of his private patients. This was L. W., a twenty-four year old unmarried woman, an extremely unstable, unmanageable diabetic. Her antecedent history was, briefly, as follows: Her diabetes was diagnosed in February 1928 when she was seventeen years of age. Moderate glycosuria was the only symptom and the diagnosis was confirmed by a glucose tolerance test. She was placed on Joslin's "C9" diet: 65 P, 95 F, 95 CHO. When glycosuria persisted, on June 1, 1928, the carbohydrate ration was cut to 60 gm. per day and she was given 25 units of insulin. Glycosuria diminished, but soon again increased, hence the insulin dose was raised to 30 units, and shortly thereafter to 35 units. As the improvement in the extent of glycosuria was always only temporary, the insulin dose was raised again and again: on November 7 to 40 units, on January 11, 1929, to 54 units. The apparent need for increasing the insulin dosage to 54 units from the initial 25 units inside a span of seven and a half months suggested to the physician a rapid "downward progress" of this patient. She was subject to hypoglycemic reactions from the outset, but these increased in frequency and severity as the insulin dosage mounted. In order to mitigate the distressing insulin shocks, in 1934 the carbohydrate ration was increased to 110 gm. and the insulin dosage was simultaneously reduced to 45 units. All this failed to prevent frequent and severe hypoglycemic reactions. At the same time it seemed impossible to check increasing degrees of glycosuria, and, as a remedy, the insulin dose was again increased to 50 units and finally to 66 units (50-0-16) daily. The patient was seriously debilitated, spent many of her days in bed. Her mother found it impossible to leave her alone in the house, after having found her several times in deep hypoglycemic shock on her return from routine shopping trips.

This was the condition of the patient when she came under our observation on October 28, 1935. Her diet was changed to 65 P, 90 F, 200 CHO, and the insulin dosage was reduced at

the outset to 40 units per day (from 66 units), distributed as 20-0-20. Her initial response to these radical changes was similar to that of the preceding four subjects; we shall not take up space, therefore, with recording here the course of her progress. Suffice it to state that on November 22 her insulin dose was reduced to 36 units (18-0-18) daily, made possible by the decrease of her glycosuria. A tendency to hypoglycemic reactions still prevailed, usually about four hours after the noon meal; to prevent these the patient was given some fruit at 3 P.M. Glycosuria, which had ranged from 52 to 103 gm. daily during the first week, fell to 33 to 51 gm. during the fourth week. On December 13 the distribution of the 36 units of insulin was changed to 20-0-16. On this regimen, during December 1936, the glycosuria showed a further decrease, ranging from 6 to 16 gm. daily. The patient thus was transferred from the status of an unmanageable diabetic to that of an easily "controlled" mild diabetic; she returned to a normal life. At this point our studies of this subject were terminated, but her physician informed us that eventually he effected some further cuts in the insulin dose. The patient has enjoyed good health, was married a year later and led a normal way of life during the next twenty years. Since 1955 we have not heard about her.

In addition to these five cases we had in 1937 a limited number of other uncontrollable, intractable cases which all responded to our use of insulin in the same manner. When these patients came under our observation they were all receiving large doses of insulin. Individual differences in their response and progress were conspicuous. For instance, D. O'C., a twenty-nine year old man who had been treated with no more than 40 units of insulin, was extremely debilitated and much painstaking work was required for his rehabilitation. However, our efforts were rewarding: currently he is using 25 units of insulin, is in good health and is the father of three children (after having been sexually impotent at the age of twenty-nine). At the other extreme, a fifty-four year old woman, who erratically used from 60 to 150 units of insulin per day, gradually became aglycosuric without the use of insulin.

AN EXPERIMENT DEMONSTRATING EXACERBATION OF DIABETES BY OVER-INSULINIZATION

The foregoing studies clearly show that it is possible to transform severe, debilitating states of diabetes to mild forms of the disease which are readily controlled by small doses of insulin (and in some cases without insulin). As our protocols show, such rehabilitation was accomplished by adjusting the insulin dosage so as to control excessive glycosuria, but at the same time taking meticulous care to avoid excess insulin action.

Is it then permissible to conclude that our subjects had become unmanageable in the first place by treatment with excessive insulin doses which caused hypoglycemic states? Perusal of the antecedent histories of our subjects justifies, we believe, an affirmative answer to this question. If one is unaware of the fact that hypoglycemia begets hyperglycemia, it appears logical to ascribe the flare-ups of glycosuria which follow in the wake of excess insulin action to insulin deficiency and, consequently, to increase the insulin dosage. This process then leads to a vicious circle and unmanageable diabetes.

In view of the obvious conflict between the conventional precepts of insulin treatment and this rather unorthodox thesis, and with regard to the practical significance of the latter, it seemed desirable to probe its validity with a closely controlled clinical experiment which could furnish conclusive, direct evidence. Any one of our rehabilitated subjects, we assumed, would revert to his former unmanageable status as a result of increasing his insulin dosage. Our Subject No. 1 (M. K.) readily volunteered for such an experiment. At this time he had been a stable, mild diabetic for an entire year and thus appeared to be an excellent subject. He was hospitalized for the experiment on August 1, 1938.

During the period of August 1 to 6, a control period to give us a base-line of reference, the patient was observed on a diet of 85 P, 325 CHO, 60 F, and an insulin dosage of 16 units per day, distributed as 5-0-6-5 units. The last 5 units were injected at 10 or 11 P.M., before a bedtime snack which consisted of one slice of bread and an apple. This was the same regimen on which the patient had been getting along excellently during the preceding whole year. As may be seen in Table VII (top section), the average urine sugar during these six days was 14.8 gm., with a maximum of 23.6 and a minimum of 6.0 gm., for a twenty-four hour period. This meant that the patient utilized 365 gm., i.e., 96 per cent of the 380 gm. glucose available in his diet, while receiving only 16 units of insulin, much the same as he had been doing for a year before this experiment.

TABLE VII
SHOWING EXPERIMENTAL EXACERBATION OF THE DIABETES OF SUBJECT NO. 1 (M. K.) BY ADMINISTRATION OF MODERATE EXCESS OF INSULIN

Date	Urine Sugar (gm.)					
	B-L	L-S	S-11	11-B	Total	Average per Day for Each Period
Insulin 16 Units (5-0-6-5) per Day Since 1/29/36						
8/1/36	1	8	3	0	12	
8/2/36	0	7	10	4	21	
8/3/36	3	7	9	5	24	15
8/4/36	0	9	0	0	9	
8/5/36	0	6	0	0	6	
8/6/36	3	4	10	0	17	
Insulin Raised to 22 Units (8-0-8-6)						
8/7/36	7	5	3	0*	15	
8/8/36	14	13	9	15	51	
8/9/36	12	20	5	0	37	
8/10/36	1	12	1	0	14	26
8/11/36	1	2	0	0	3	
8/12/36	9	6	3	0†	18	
8/13/36	15	7	14	7	43	
Insulin Raised to 24 Units (8-0-10-6)						
8/14/36	13	2	0	0†	15	
8/15/36	14	15	11	7	47	
8/16/36	11	18‡	8	0‡	37	
8/17/36	11	21	0.2	2‡	34	44
8/18/36	14‡	22	13	9	58	
8/19/36	32	18	9	5‡	64	
8/20/36	17	18	15	1†‡	51	

* Severe hypoglycemic reaction at 10 P.M.
† Severe hypoglycemic reaction between 11 P.M. and midnight.
‡ = Ketonuria.

As the next step, we simulated the conventional procedure, setting as a goal the complete suppression of glycosuria, by adding 6 units to the insulin dosage, making it 22 units per day, distributed as 8-0-8-6 units. On the very first day of this change the patient had a hypoglycemic reaction at midnight and, as may be seen in Table VII (middle section), the glycosuria next day increased sharply. Another pronounced reaction was observed on the sixth day (August 12), with an adverse aftermath as before. During seven days on the increased insulin dosage, *the average daily urine sugar was 25.7 gm.*, twice as much as on the previous lower dosage, with a minimum of 2.8 and a maximum of 50.5 gm.

TABLE VIII
SHOWING EXTREME OSCILLATIONS OF THE GLYCEMIC LEVEL IN SUBJECT NO. 1 (M. K.) AS A CONSEQUENCE OF OVER-INSULINIZATION

Date	Time	Mg. Sugar per 100 cc. Blood
8/14/38	4:30 P.M.	117
	10:00 P.M.	26
8/15/38	6:30 A.M.	331
8/20/38	Midnight	80
8/21/38	3:00 A.M.	28
	7:00 A.M.	234
11/14/38	3:30 A.M.	54
	7:00 A.M.	169

As the glycosuria increased, once more we followed the conventional procedure by adding on August 14 another two units to the dose before supper, raising the total to 24 units per day. As may be seen in the third section of Table VII, as a consequence of this second increase in dosage the patient's condition suffered further deterioration: in the course of one week the glycosuria mounted to an average of 40.8 gm. per day, with a maximum of 63.7 gm. Even more disturbing was the emergence of ketonuria on August 16, the third day after the insulin dosage was raised to 24 units.

The causal connection between excess insulin action and exacerbation of the diabetic syndrome is shown clearly enough by the data given in Table VII, but for added documentation we performed a limited number of blood sugar determinations, a few examples of which are recorded in Table VIII. As may be seen, during the night the blood sugar would fall to as low as 26 mg. per cent, which then entailed "fasting" hyperglycemic levels as high as 331 mg. per cent. Such flare-ups in the morning were usually accompanied by pronounced ketonuria.

The "downward progress" of our subject in response to excess insulin action, so strikingly shown in the last column of figures in Table VII, developed at an unexpectedly rapid pace: an excess of only 8 units of insulin put him on the path leading back to the state of severe, unmanageable diabetes which he had endured for years prior to his rehabilitation. Alarmed by our

TABLE IX

REHABILITATION AFTER EXPERIMENTAL EXACERBATION OF DIABETES

Date	Urine Sugar (gm. per 24 hr.)		Insulin (units)		Diet
	Average	Range of Fluctuations	24 hr.	Distribution*	
10/2/38–10/12/38	40	32 –50	19	10-0-6-3	The diet was 80 P, 70 F, 275 CHO
11/5/38–11/13/38	22	16 –26	21	8-0-8-5	During the 10/2–10/12 period, 25 gm. extra sugar was given at 10 A.M. and 1 slice bread with a 120 gm. apple at 11 P.M.
11/14/38–11/22/38	20	15 –26	21	8-0-8-5	
12/2/38–12/11/38	10	4 –14	19	8-0-8-3	
12/12/38–12/21/38	5	0.6–11	19	8-0-8-3	During the subsequent intervals, the 10 A.M. sugar was cut to 12 gm. and the apple was omitted at 11 P.M.
12/22/38–12/31/38	4	0 –12	16	8-0-8	
1/1/39–1/10/39	4	0 – 9	16	8-0-8	

* Where a fourth dose is indicated, it was given at 11 P.M.

"success," more by the ketonuria than by the increase in glycosuria, we terminated this phase of the experiment and set out to restore the patient's condition to the mild, stable condition which had prevailed for a year preceding this experiment.

Excerpts from our daily protocols, given in Table IX in condensed form, are sufficiently descriptive of the procedure that was followed, without requiring comment. For added information, in Table X are presented detailed data for a ten-day period. It may be gleaned from the two tables that this second course of rehabilitation carried the patient to a better balance and stability than had been attained prior to the experimental exacerbation of his diabetes. He ended up on two instead of three doses of insulin, 8 units each, and his glycosuria was reduced below the previous best level. As may be seen in Table X, during a ten-day period two days were completely aglycosuric, and on another three days only the night urine contained a small amount of sugar. Compared with his condition prior to the experiment (see the period August 1 to 6 in Table VII), the average of daily urine sugars now was as low as 4.4 gm., as against 14.8 gm., and the day-to-day fluctuations narrowed down from a range of 6.0 to 23.5 to 0 to 11.5 gm., a considerable improvement and progress in the direction of stability.

TABLE X

SHOWING THAT DAY-TO-DAY AS WELL AS DIURNAL FLUCTUATIONS OF GLYCOSURIA IN SUBJECT NO. 1 (M. K.) WERE SLIGHT AFTER SECOND REHABILITATION

Date	Urine Sugar (gm.)				
	B-L	L-S	S-11	11-B	Total
Insulin 16 Units (8-0-8) per Day					
12/22/38	0	0.7	0.6	3.4	4.7
12/23/38	1.1	2.0	1.4	7.0	11.5
12/24/38	1.3	0.6	0.5	0	2.4
12/25/38	0	0	0	0	0
12/26/38	0	0	0	2.1	2.1
12/27/38	0	0	0	0	0
12/28/38	0	0	0	6.3	6.3
12/29/38	1.8	1.3	0.6	3.6	7.3
12/30/38	0	0	0	4.0	4.0
12/31/38	0.6	1.4	1.8	1.9	5.7

This experiment concluded three and a quarter years of close studies on Subject No. 1. We are persuaded that these studies furnish direct evidence to the effect that *a mild state of diabetes can readily be shifted into a severe state of the disease under the influence of excessive insulin action, and, furthermore, that the process is reversible.*

CORROBORATION BY OTHER WORKERS

In May 1938 the facts described in the section "Observation on Unmanageable Patients" were presented at a meeting of the St. Louis Medical Society and published in the Weekly Bulletin of the Society [6]. This report aroused the interest of a number of younger men who in several local hospitals began to apply our findings to the insulin treatment of their patients, invariably

with gratifying results. The first such result was brought to our attention by Dr. J. R. Ready, who was in our audience at the Medical Society meeting. His father was in a hospital in another city, unsuccessfully "regulated" with large doses of insulin. Dr. Ready immediately traveled to the bedside of his father and—with no actual experience in the field (he was medical director of a life insurance company)—took management into his own hands. We deem it of sufficient interest to reproduce here pertinent parts of the hospital records of this case:

In May 1938, A. J. R., a sixty-eight year old man, was "admitted to hospital in insulin shock. History of being a diabetic; last saw physician two years ago . . . staying on no particular diet . . . has been taking 20 units insulin three times a day; on day of admission had done some extra hard work in the forenoon . . . admitted at 7:40 P.M." in a state of insulin shock. "Given 25 gm. glucose I.V. . . . The next day was started on diet: carbohydrates 150; protein 85; fat 150 with insulin units 20, three times a day. Blood sugar taken each morning and afternoon . . . ranged up to 230 mg. . . . his insulin was increased to 25 units three times a day . . blood sugar 91 mg. in the evening and 286 mg. in the morning. Insulin dosage increased in number to four . . . 25 units to be given at midnight" (a total of 100 units per day).

At this point Dr. Ready intervened and "a radical change in diet and therapy was instituted and he was put on . . . 200 gm. carbohydrate, 100 gm. protein, 65 gm. fat . . . Insulin was discontinued. Blood sugars were as follows: 5/15 A.M., 200 mg.; 5/16, 154 mg.; 5/17, 118 mg.; 5/18, 133 mg." The urine became free of sugar and acetone. "Was completely free of symptoms . . . Blood count essentially normal . . . discharged from the hospital . . . without insulin."

(It must be stated at this point that abrupt elimination or even substantial reduction of the insulin dosage is not advisable, as it can invite very serious trouble. Such changes should be made gradually, with guidance by careful quantitative checks in the laboratory.)

The further course of this patient was excellent. A glucose tolerance test with 100 gm. oral glucose, performed in our laboratory on January 10, 1941, when he was seventy years old, gave the following result:

	F	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood sugar, mg. %	107	183	257	215	175	101
Urine sugar	0	0	trace	0.9 gm.	1.0 gm.	trace

This "diabetic" patient is now eighty-eight years old. He lives on a well balanced diet, containing 65 to 70 gm. fats; he is enjoying good health and retired from business only two years ago.

As early as 1938 a representative member of the group that collaborated with us, Dr. W. F. Friedewald, then chief resident of the St. Louis City Hospital, presented before the St. Louis Medical Society a series of case reports, with the conclusion: "The principles which Dr. Somogyi has brought forth have been entirely substantiated. In general there has been a marked improvement in the management of our diabetic patients." Elimination of hypoglycemic reactions, Dr. Friedewald added, resulted in better regulation and stabilization of patients on substantially decreased insulin doses. Three of his patients had been severe unmanageable diabetics for a number of years. "These patients," Dr. Friedewald reported, "are now well controlled, with a marked reduction in the total insulin dosage and they receive a practically unrestricted diet" [*7*].

In a case described by Berger [*8*], a private patient, not previously treated with insulin, had a fasting blood sugar of 210 mg. per cent. In the Jewish Hospital his private physician started him off on 30 units a day, distributed in three equal doses. On the second day under this management, the fasting blood sugar increased to 252 mg. per cent, and in consequence the insulin was raised to 50 units; and when this did not bring the fasting blood sugar down, the insulin dosage was gradually raised to as much as 100 units a day. The patient became unmanageable, had severe insulin shocks and often required the intravenous administration of glucose for his recovery. At this point Dr. Berger, resident physician, took over and in the course of two weeks reduced the insulin dosage to 12 units a day, on which the patient was aglycosuric, whereas on the high insulin dosage aglycosuric days were followed by days with marked glycosuria. As the insulin dosage was rapidly lowered, the fasting blood sugar also decreased to 205 mg. per cent, the lowest level during these observations. The patient was discharged by his physician before further progress could be made.

Bowcock [*9*], in 1939, was the first clinician outside our local group who reported favorable results attained by the clinical application of our principles. Herold [*10*] likewise found the application of our principles of considerable value.

Lavietes and Peters [*11*] also took cognizance of our thesis and applied it in the treatment of numerous diabetic patients. After sufficient clinical observation, they reported that insulin reactions cause exacerbation of the diabetic syndrome by giving rise to increasing hyperglycemia and glycosuria, as well as ketosis. They reported excellent clinical results by "diligent search for hypoglycemic symptoms" and by taking appropriate measures to avert them. Later, Burns [*12*] reported successful application of this principle. He described cases in which he could greatly reduce the insulin dosage, and others in which insulin injections could be entirely eliminated. More recently Goodman [*13*] obtained favorable results by observation of our thesis that "hypoglycemia begets hyperglycemia." By arranging the dietary regimen of his patients in a manner that helped to prevent hypoglycemic reactions (intermediate feedings), he succeeded in preventing excessive glycosuria while administering only moderate doses of insulin. More recently, Perkoff and Tyler [*14*] reported substantial improvement in the condition of patients with severe diabetes by following our precepts in their treatment. By avoiding hypoglycemic states they eliminated, or at least mitigated, "paradoxical hyperglycemia" and "cyclic glycosuria," which are unmistakable sequelae of over-insulinization. Before they resorted to such a course their patients showed "deterioration of diabetic regulation on increasing insulin doses." From personal communications we are informed that a number of other workers, who have not published their experiences, were equally successful in improving the condition of unstable diabetics by careful application of the rule that prevention of hypoglycemic states is of no less importance than efforts to keep glycosuria as low as possible.

Members of the group with which I collaborated have, in the course of years, treated hundreds of patients with severe and unmanageable diabetes. In time the management of these patients became simpler as experience and better understanding of the physiologic laws involved greatly reduced the element of error in a procedure that at the outset was mostly a process of learning by trial and error. Interesting examples of these cases will be described in separate reports by individual members of our team of collaborators. In the meantime it may be stated that the results in every one of these many cases were as favorable as in the cases we have described.

INTERPRETATION

The foregoing studies exposed hypoglycemia as the cause of the perplexing fluctuations of the glycemic level and glycosuria commonly observed in diabetic patients treated with insulin. This makes it understandable why the amount of carbohydrates utilized per unit of insulin (G/I ratio) decreases when the ratio R (= available glucose/insulin dosage) is depressed: the larger the insulin dose in relation to the amount of the ingested carbohydrates, the greater are the chances for development of hypoglycemic states, which then entail the defeat and frustration of insulin action. This explains why Allan's depancreatized dog utilized 20.5 gm. glucose per unit of insulin with a dose of 4 units, but only 1.5 gm. when the dosage was increased to 30 units.

With the causal connection between excess insulin action and its hyperglycemic-glycosuric aftermath clearly documented by empirical experiments, it was inevitable that we should turn our attention to the mode of action of hypoglycemia, to the physiologic processes underlying its diabetogenic effect. We found that the literature of the past three and a half decades shed generous light on the problem, and it also revealed that what we have accomplished in our studies was nothing more than incorporation of long known physiologic laws in the application of insulin therapy.

Physiological studies, published from 1924 to the present day, supply unequivocal evidence to the effect that insulin hypoglycemia stimulates the adrenal-pituitary system to an accelerated release of blood sugar raising hormones. Whenever the blood sugar falls to a "critical level" (Cannon), it elicits, with a trigger-like, instantaneous effect, an increase in the rate of adrenalin secretion, a reaction which is stimulated via the splanchnic nerves. Adrenalin then excites the anterior pituitary to an increased secretion of ACTH, and this, in turn, elicits increased adrenocortical activity. Other complex processes may be involved, but discussion of these details is irrelevant in the present context; it suffices to know that insulin hypoglycemia mobilizes adrenalin and other hormones which raise the glycemic level and hence are often referred to as *insulin antagonistic* or *anti-insulin* factors.

The effects of insulin hypoglycemia on the adrenal-pituitary system were demonstrated, mostly in laboratory animals but also in human subjects, by various experimental methods. Earlier workers employed technics of bioassay in gauging quantitative changes in the adrenalin content of blood; later on chemical methods were used for the estimation of adrenalin and of corticoids. Valuable observations were made on morphologic changes (hyperplasia, hypertrophy) of the adrenal and pituitary glands which were induced by insulin hypoglycemia.

Houssay and his associates [*15*] in 1924 were among the first to prove that insulin hypoglycemia accelerates adrenalin release in the dog; they regarded a rapid rise of the blood sugar to hyperglycemic levels as evidence of increased adrenalin action. In their experiments the suprarenal vein of the donor, a large dog, was anastomosed with the jugular of the recipient, a small dog, and transfusion was started when the blood sugar of the donor had fallen to 30 to 40 mg. per cent, one hour after insulin injection. The blood sugar of the recipient, determined from then on at fifteen minute intervals over a period of two to three and a half hours, rose considerably, in one instance as much as 190 mg. per cent above the initial fasting level. When the splanchnic nerves of the donor were transected directly after the injection of insulin, no hyperglycemia was produced in the recipient. Houssay considered his findings as conclusive evidence of increased secretion of adrenalin under the impact of insulin hypoglycemia; he found that the rate of adrenalin secretion had to increase to at least sevenfold the normal rate in order to produce hyperglycemias such as were observed in the experiments.

About the same time Cannon et al. [*16*] demonstrated the increase in the rate of adrenalin secretion during insulin hypoglycemia by quite a different method. They showed that after injected insulin had lowered the blood sugar to hypoglycemic levels, the heart rate was accelerated in the same manner as by the injection of adrenalin; and the lower the hypoglycemic level, the greater was the increase in the heart rate. The first measurable change in rabbits occurred when the descending blood sugar level reached 80 mg. per cent, the "critical level." (It is to be noted that this sugar value represents the total reducing matter in a Folin-Wu blood extract, which is 15 to 20 mg. per cent higher than the true glucose content. Thus the actual critical level was 60 to 65 mg. per cent.) When the splanchnic nerve was severed, insulin hypoglycemia had no effect on the heart rate.

Almost simultaneously, other investigators described profound morphologic changes in the adrenal glands under the influence of insulin hypoglycemia. The first such observation came in 1924 from Riddle et al. [*17*], who reported that in pigeons "poisoned" with insulin the adrenals were enlarged by 50 to 100 per cent above the normal size, the change involving both parts of the gland. Besides this, the blood sugar fluctuated irregularly, rising from hypoglycemic levels as low as 25 mg. per cent to abnormal hyperglycemic peaks, well above the initial level. The authors attributed these effects of "insulin poisoning" to increased activity of the adrenal glands and made the interesting remark: "*These results supply an additional reason for avoidance of heavy insulin dosage in man.*" Poll [*18*] in 1925 described similar changes in mammals. After "insulin intoxication" the histologic picture of the adrenal gland, he reported, is completely changed, the changes becoming increasingly greater with prolongation of the toxic state. The cortex is vacuolized, the structural elements show "a state of confusion"; in the medulla the "adrenalogenic" (chromaffin) cells are scarcely identifiable, appear exhausted, show "*histologic signs of an outpouring of adrenalin.*" In 1926 Kahn [*19*], in extensive experiments with mouse, dog and rabbit, confirmed Poll's findings, stating that "insulin intoxication" wreaks profound morphologic changes in both parts of the adrenal gland. Most conspicuous is the exhaustion of the chromaffin cells, not only in the adrenal medulla but also in the paraganglia along the abdominal aorta. Along with these changes the adrenalin content of the glands is diminished and virtually exhausted if the hypoglycemic state is protracted. Transection of the splanchnic nerves protects the adrenal medulla against the changes wrought by insulin hypoglycemia. It follows from this, states Kahn, that we are confronted with "a centrally attacking action of insulin intoxication."

During the ensuing three decades a large number of exhaustive and interesting studies of this subject were published, experiments carried out on a variety of laboratory animals, with modern technics, including refined chemical methods of analysis. They all confirm the find-

ings of the pioneers in the field, but also contribute additional pieces of valuable information. We shall forego references to these articles and look into some interesting reports dealing with human subjects.

Boothby and Wilder [*20*] were the first investigators to observe an abnormally accelerated release of adrenalin in a diabetic person during a mild degree of insulin hypoglycemia; as a matter of fact these observations were reported in 1923, before the studies on laboratory animals, outlined in the preceding paragraphs, were published. In an experiment, a patient was given 20 units of subcutaneous insulin in the postabsorptive state, and changes in the blood sugar, respiratory quotient and metabolic rate were measured for several hours. In two hours the blood sugar dropped steeply to 143 mg. from an initial 286 mg. per cent, then to 90 mg. per cent during the next eighty minutes (this value corresponds to 60 to 70 mg. per cent true sugar). The results, as well as the symptoms, were identical with those produced by the injection of adrenalin. The same effects were even more pronounced in a healthy subject, when his insulin hypoglycemia fell as low as 40 mg. per cent. The data, the authors concluded, "and the other phenomena observed are so similar to those after the administration of adrenalin that the possibility of a *spontaneous discharge of epinephrine must be considered.*"

The bearing of this observation on insulin therapy was, it seems, not appreciated. Wilder himself considered insulin hypoglycemia innocuous as long as it caused no serious reactions, stating, four years later, in one of his books [*21*]: "*Mild reactions* which every patient taking insulin soon learns to recognize, *are by no means injurious and may be even beneficial.*" (Italics ours.)

Among recent workers, Holzbauer and Vogt [*22*] showed accelerated secretion of adrenalin elicited by hypoglycemia in man and dog. The man was given intravenously 0.24 units of insulin per kilo body weight, and the ensuing changes in the sugar and the adrenalin level of the plasma were repeatedly measured. It was found that the adrenalin concentration increased measurably when the plasma sugar declined to about 75 mg. per cent, and rose to the *thirtyfold of the initial* (normal) level when it dropped to 40 to 45 mg. per cent. Sugar was determined by the Hagedorn-Jensen method, which yields values 10 to 15 mg. per cent higher than true sugar; hence the 75 mg. per cent recorded by these authors represents 60 to 65 mg. per cent true sugar, interestingly, the same as Cannon's "critical level." In graphic presentation of the data of these experiments, the sugar and adrenalin curves appear as mutual mirror images, a picture also obtained by other workers.

More recently, investigators assayed changes in the corticosteroid level in blood and urine as an effect of insulin hypoglycemia. These changes, as is known, run parallel with changes in the adrenalin level, as excess of the latter triggers the accelerated release of ACTH and, consequently, of corticosteroids. We quote the results of a few such studies. Klein et al. [*23*] found in diabetic children treated with insulin significantly higher free serum corticoids than in normal children. Bliss et al. [*24*], in their studies of mental patients treated with insulin shock, stated: "Significant increases in the concentration of the 17-hydroxycorticosteroids in the blood occurred on every occasion with insulin coma." An extensive study by McArthur et al. [*25*] on a diabetic patient included the determination of corticosteroids excreted in the urine after the injection of variable amounts of insulin, ranging from 25 to 80 units. One of their observations is of especial interest: "*The administration of large doses of insulin,*" they stated, "*in attempts to insure continuous normoglycemia resulted in the occurrence of frequent insulin reactions and in measurable increases in the rate of corticostreroid excretion.*" On the other hand, they found that "*the administration of small doses which just prevented ketonuria exerted no detectable effect upon the rate of corticosteroid excretion . . . .*" (Italics ours.)

The literature abounds in reports supplying unequivocal evidence to the effect that insulin hypoglycemia accelerates the release of adrenalin, ACTH and corticosteroids. These reports are so unanimous that the few examples quoted suffice to put in focus the physiological reactions which are set off by hypoglycemia, and fully explain the results obtained in our clinical observations.

The use of the term, hypoglycemia, requires clarification at this point. In general usage a subnormal blood sugar level is not referred to as hypoglycemia as long as it does not manifest itself in certain symptoms (which need not be described here). For proper understanding of the intricacies of insulin action, however, it is imperative to adhere to the physiologic definition, i.e., *to consider as hypoglycemia any blood sugar value that is measurably below the normal postabsorptive*

level, for even a fall of a few milligrams per cent below this level suffices to accelerate the release of insulin antagonistic factors.

In consideration of the practical significance of the thesis that excess insulin action can seriously impair the carbohydrate tolerance of human subjects, it appeared desirable to adduce added evidence in support of its validity. Direct evidence, we believed, could be best obtained by experimental reproduction of the effects of insulin hypoglycemia; this principle was followed in an experiment performed on Subject No. 1 and described in the section "An Experiment Demonstrating Exacerbation of Diabetes by Over-insulinization"; but reluctant to subject other diabetic patients to similar protracted experiments, we devised several other approaches and methods.

In one series of experiments the changes in the A-V difference were measured to show that even mild degrees of hypoglycemia suffice to elicit insulin antagonistic reactions. An example, taken from an earlier paper [*26*], will illustrate this physiologic process. As may be seen in Table XI, in this subject a mild degree of hypoglycemia developed, 61 mg. per cent true sugar (this would be about 75 mg. per cent if determined by the Folin-Wu method), one hour after the injection of insulin; then the blood sugar took a sharp upturn, despite the fact that insulin at that point still acted with maximum intensity. But insulin action was evidently countermanded and outstripped by the action of its antagonists which were released at an accelerated rate under the stimulus of hypoglycemia. Both the rise in the blood sugar level and the abrupt shrinking of the A-V difference are characteristic manifestations of increased adrenalin action; this fact we have demonstrated in several other experiments [*27,28*].

In another type of experimental approach in our studies, the diabetogenic action of hypoglycemia was demonstrated by its effect upon the glucose tolerance of healthy and of diabetic subjects. Mild hypoglycemic states of brief duration were produced by intravenous injection of 4 or 5 units of insulin, and forty minutes after the injection the subjects were fed 100 gm. of glucose. No perceptible hypoglycemic symptoms occurred, except in one subject, a diabetic who received 7 units; this man perspired and felt tremors in his hands at the time he ingested the glucose. Yet the blood sugar time-curves showed that such short and mild hypoglycemic

TABLE XI

SHOWING MANIFESTATIONS OF ADRENALIN ACTION, ELICITED BY INSULIN HYPOGLYCEMIA

Hours after Insulin	Mg. Glucose per 100 cc. Blood		A-V Difference (mg.)
	Arterial	Venous	
0	205	203	2
0.5	123	82	41
1	80	61	19
1.5	100	97	3
2	136	134	2
3	151	149	2

states sufficed to cause marked deterioration of the glucose tolerance in every one of the subjects. One healthy man even showed glycosuria in the experiment. In diabetic subjects the same response to hypoglycemia took place on a markedly magnified scale. In one of the subjects, who received 7 units of insulin, and glucose 75 minutes after the insulin injection, the diabetic state suffered serious exacerbation: although he had been consistently aglycosuric for about one year without using insulin, the hypoglycemic incident precipitated greatly increased hyperglycemia, massive glycosuria and ketonuria. Temporary insulin treatment became necessary to combat the disturbance, and even so, five weeks elapsed before restoration to his previous condition was accomplished by arduous, day-to-day efforts [*29*].

A third series of experiments [*30*] demonstrated the fact that hypoglycemia elicits pronounced resistance to insulin action in healthy men, after the glycemic level has been depressed to at least 60 to 65 mg. per cent ("critical level"). In one subject intravenous injection of 4 units of insulin lowered the blood sugar by 46 mg. per cent, but when the insulin supply was boosted by a second 3-unit dose, injected forty-five minutes after the first, only a 19 mg. per cent fall was produced.

In an extension of this line of experimentation, we had to resort to the use of laboratory animals. These experiments revealed that in rabbits subjected to periodic, severe insulin reactions for several months, resistance to insulin action developed to such a degree that subcutaneous insulin doses of 11 units/kg. produced but an insignificant, virtually negligible, decline in the

blood sugar, whereas they were thrown into severe convulsions, with hypoglycemic levels as low as 20 mg. per cent, by the subcutaneous injection of 1.5 units/kg. during the first week of the experiment [*31*].

The physiological reactions involved in the foregoing experiments are, as is known, in continual operation in the healthy organism as integral components of the homeostatic mechanism which keeps the blood sugar level on an even keel. Under *normal* physiological conditions hypoglycemic intervals are slight of degree, in fact scarcely measurable by analytical methods, and are short of duration, owing to the delicately dynamic response of the adrenal-pituitary system; the decline of the blood sugar level is halted long before it can progress to anything near a "critical level." But the situation is different when excess action of injected insulin causes hypoglycemia. Such hypoglycemias fall to and below the critical level and often persist over prolonged periods of time. This is a clear reflection of the fact that the capacity of the adrenal-pituitary system is unable to cope with the demand for blood-sugar-raising hormones, and *insulin hypoglycemia is placed in the category of stressor agents; the ensuing hyperglycemic-glycosuric tide, then, is an alarm reaction in response to the stress.* This facet of the problem of insulin therapy has in recent years engaged the attention of several investigators; it requires and merits separate discussion.

SUMMARY

1. Detailed laboratory data gathered on severe diabetic subjects, in closely controlled observations and experiments, show complete lack of parallelism between the amount of insulin injected and the amount of carbohydrates utilized; even when both diet and insulin dosage are kept constant, glycosuria varies over wide ranges and the blood sugar oscillates between very low and very high levels.

2. The laboratory records of patients who are treated with substantial doses of insulin reveal a consistent pattern of periodic ebb and tide in the fluctuations of both the glycosuria and the glycemic level, and disclose the fact that the high tides consistently occur in the wake of hypoglycemic reactions, even after asymptomatic, mild degrees of hypoglycemia. Thus a clear-cut cause and effect relationship unfolds itself between hypoglycemia and the ensuing upsurge of hyperglycemia, and we are confronted with the paradoxical fact that *excess insulin action can produce hyperglycemia.* It can be stated that, barring other physiological and emotional stresses, *conspicuous fluctuations in glycosuria are unmistakable indicators of excess insulin action.*

3. The impairment of carbohydrate tolerance as a sequel of hypoglycemia is readily explained on the basis of experimental evidence available in the literature and supplemented by studies in our laboratory, which show that hypoglycemia elicits an accelerated release of adrenal-pituitary blood-sugar–raising hormones. Increase in the depth and duration of the hypoglycemic state intensifies the stimulus upon the secretory activity of the adrenal-pituitary system; as a consequence the action of the blood-sugar–raising hormones can cancel out the action of injected insulin and tip the balance in favor of the former. The result is a sharp rise in the glycemic level despite the presence of active insulin, and exorbitant hyperglycemia and glycosuria after alimentation in this condition.

4. When hyperglycemia due to excess insulin action is countered by increasing insulin doses, under the assumption that it can only result from a deficient insulin supply, the result is exacerbation of the diabetic syndrome, manifested in extreme fluctuations of glycosuria, with mounting peaks and ketonuria; at the same time, recurrent hypoglycemic reactions increase in severity.

5. It is evident that in insulin therapy, avoidance of hypoglycemia, even of mild, asymptomatic degrees, is no less important than the control of excessive hyperglycemia and glycosuria. Application of this precept makes it possible to forestall the development of severe states of diabetes, and to restore patients with severe diabetes to a status of mild diabetes that can be satisfactorily managed with small doses of insulin or, not infrequently, without insulin.

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Diabetogenic Effect of Hyperinsulinism*

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PERSONS afflicted with hyperinsulinism, due to islet cell tumors of the pancreas, commonly show impaired carbohydrate tolerance, inasmuch as they respond to glucose tolerance tests like diabetic patients. Another abnormality they exhibit consists of extreme fluctuations of the blood sugar between hypoglycemic and diabetic-like hyperglycemic levels. A third manifestation of their disturbed carbohydrate tolerance is a transitory diabetes following extirpation of the offending tumor.

Virtually identical disturbances occur in healthy persons who are treated with insulin injections and are thereby placed in a state of artificial hyperinsulinism. In the present paper we offer an interpretation of these paradoxical phenomena which, as far as we are aware, are still in want of satisfactory explanation.

ORGANIC HYPERINSULINISM

The term, organic hyperinsulinism, is employed here for patients who were proved to have derived an excessive insulin supply from pancreatic tumors involving the islets of Langerhans. As a direct proof of hyperinsulinism, in the celebrated case of Wilder et al. [*1*], considerable amounts of insulin were extracted not only from the primary tumor, but also from the metastatic tissues found in the liver. The man died when continuous infusion of huge amounts of glucose failed to raise his blood sugar above deep, hypoglycemic levels. Yet—and this is the salient point—in a glucose tolerance test performed on this patient a short time before his death, hyperglycemia rose to a peak (283 mg. per cent) as high as in diabetes. At an earlier stage of his illness the patient was actually treated for diabetes. Flinn et al. [*2*] had a case virtually identical with Wilder's, an islet cell carcinoma with massive metastasis in the liver and fatal outcome. This patient, too, showed greatly diminished glucose tolerance a short time before his death. In a third fatal case, reported by Hartmann [*3*], during a glucose tolerance test, the rise in blood sugar was as high as in diabetes. In numerous other cases reported in the literature the patients were restored to health by surgical removal of the tumors which produced excessive amounts of insulin; but during the state of hyperinsulinism these patients showed hyperglycemic peaks characteristic of diabetes.

This paradox must have puzzled several authors, but failed to elicit from them any explanation. The only pertinent comment we have come across was offered by Sevringhaus [*4*]. He suggested that the diabetic-like glucose tolerance "is probably to be explained by the over-filling of the glycogen stores under the feeding used to offset the symptoms" (i.e., hypoglycemic reactions); he believed, in other words, that the glucose absorbed in a tolerance test piles up in the blood because there is not enough space for glycogen deposition in the tissues, which are "over-filled" with glycogen, owing to high carbohydrate diet and intermediate feedings. This idea, however, clashes with the fact that in many instances the tolerance curve terminally declines sharply to hypoglycemic levels, at a time when a new batch of glycogen has been deposited on top of the existing stores.

Sevringhaus' theory is further disproved by a little experiment we performed on a patient who had an islet cell adenoma. The patient was subjected to two glucose tolerance tests separated by four days. The first was a conventional test with 100 gm. oral glucose; the second differed solely in that the patient was fed two slices of bread and a cup of milk two hours preceding the test. The results were as follows:

	Blood Sugar (mg. per 100 cc.)				
Hours after glucose feeding	0	½	1	2	3
1st test	53	141	199	121	65
2nd test	73	120	151	101	44

* From the Jewish Hospital of St. Louis, St. Louis, Missouri.

As may be seen, the second test showed distinctly better ability to transfer glucose into the tissues after a preliminary load of about 40 gm. of carbohydrates.

At the root of the trouble, we submit, lies the diabetogenic effect of hypoglycemia that is produced by excess insulin action. We have given detailed consideration to this subject in a preceding paper [*5*], citing factual evidence to the effect that insulin hypoglycemia accelerates the release of blood-sugar–raising hormones from the adrenal-pituitary system, setting off a running contest between the latter and excessive insulin action. Under the recurrent stress of hypoglycemia, the insulin-opposing factors can gain ascendency over insulin action and thus can produce hyperglycemia despite hyperinsulinism. Scanning the literature, we find that in postabsorptive states hyperglycemia during hyperinsulinism occurs but seldom; this is so because the amount of glucose that is available to enter the blood stream is limited on one hand by the secretory capacity of the insulin-opposing glands, on the other hand by the rate of hepatic glycogenolysis (plus gluconeogenesis); hence the fasting blood sugar is mostly hypoglycemic or, in some instances, normal. When, however, a glucose tolerance test is performed in this condition and the digestive tract joins the liver in supplying sugar to the blood, the glycemic level can rise to diabetic-like heights; *this is a manifestation of a transitory preponderance of the insulin opponents over insulin.* During the hyperglycemic phase the stimulus on the adrenal-pituitary system is eliminated, the release of insulin-opposing factors subsides, and insulin action once again becomes predominant, as manifested in the hypoglycemic termination of the tolerance curve.

There are, however, cases in which the tolerance test showed no diabetic characteristics. This apparent conflict can be explained, we believe, by the continually changing quantitative relationship between the two rival forces, the action of insulin and that of its opponents. We applied this interpretation to eight individual tolerance tests compiled by Wilder [*6*]. This series of cases embraces four discernible types of curves, which are so grouped and interpreted in Table 1.

This interpretation is supported by the fact that in several cases in which repeat tolerance tests were performed during the state of hyperinsulinism, one test yielded a diabetic-like curve, while the other did not. Such findings were reported by Wilder et al. [*1*], Howland et al. [*7*], Isaacs [*8*] and several other workers. The two tolerance tests of our own patient are a case in point: in the first test the hyperglycemic peak was abnormally high, indicating that the blood-sugar–raising factors asserted their action in opposition to insulin, whereas in the second test insulin action gained ascendency because the release of adrenal-pituitary hormones was slowed down during the two hours preceding the test, owing to the elimination of the stimulating effect of hypoglycemia.

TABLE 1

INTERPRETATION OF SEVERAL ABNORMALITIES OF GLUCOSE TOLERANCE IN THE STATE OF ORGANIC HYPERINSULINISM

Type of Glucose Tolerance Curve	Interpretation
1. In two cases: Fasting blood sugar normal, hyperglycemic peak over 200 mg. per cent	Insulin-opposing factors already predominated at start of test
2. In three cases: Fasting blood sugar below 50 mg. per cent, no diabetic-like peak	Insulin supply outstripped the rate of release of opposing hormones
3. In two cases: Hypoglycemic fasting level, followed by hyperglycemic peak within the normal range	Action of excess insulin approximately balanced by adaptation reaction
4. In one case: Fasting blood sugar 130 mg. per cent, which remained the highest point of the curve. (On other days fasting blood sugar was below 50 mg. per cent)	On this occasion the secretory capacity of the adrenal-pituitary system was high enough to outstrip during the early morning hours the effect of hyperinsulinism, so that hyperglycemia in the fasting state ensued. During the hyperglycemic phase excitation, hence overactivity, of the adrenal-pituitary system subsided, thus insulin action again became predominant

One more abnormality connected with hyperinsulinism often reported is the fact that a state of transitory diabetes follows elimination of the excess insulin supply by extirpation of the offending tumor. Howland et al. and Womack et al. [*7,9*], for instance, obtained diabetic-like glucose tolerance tests and observed glycosuria in their cases over periods of sixteen to twenty-two days after the operation. But the recovery of normal carbohydrate tolerance may take place in a shorter period, as was the case in a patient of Lukens and Ravdin [*10*]. A glucose tolerance test performed during the state of hyperinsulinism presented a distinct picture of diabetes, but a second test, on the ninth postoperative day, gave perfectly normal results; hyperglycemia-glycosuria had persisted for only a few days after the operation. "The transitory diabetes," commented the authors, "has been

TABLE II
SHOWING DETERIORATION OF CARBOHYDRATE TOLERANCE AS CONSEQUENCE OF ARTIFICIAL HYPERINSULINISM

Experimental Conditions	Blood Sugar (mg. per 100 cc.)*				
	0 hr.	½ hr.	1 hr.	2 hr.	3 hr.
Case of Wilder et al.					
Before insulin treatment	89	111	146	111	101
After 12 days of insulin injections (up to 55 units per day)	115	149	194	250	231
After 1 day without injections	99	155	178	196	168
After 3 days without injections	83	125	151	157	125
Case of Clark et al.					
Before insulin treatment	87	159	126	95	...
After 14 days of insulin injections (up to 30 units per day)	87	182	231	100	...
After 31 days of insulin injections (up to 75 units per day)	87	208	231	204	...
After 5 days without injections	114	204	208	99	...
After 8 days without injections	91	186	173	109	...
Case of Maher and Somogyi					
Before insulin treatment	74	145	124	63	47
After 6 months of insulin injections (10 to 20 units per day)	74	176	318	218	107
After another 6 months (20 units per day)	93	...	329	308	154

* Hours after glucose feeding.

observed by others and is presumably a phenomenon of readjustment."

For a fuller understanding of the nature of the transitory diabetes and subsequent recovery from it in these cases one must be aware of two separate adaptation reactions involved. The first of these takes place during the preoperative state when, over long periods of time, often years, frequent hypoglycemic attacks (stressor agent) excite the adrenal-pituitary (blood-sugar–raising) system to exaggerated activity; this accounts for the diabetic-like response in the preoperative glucose tolerance tests. When surgery suddenly eliminates the stimulus exerted by excess insulin action, overproduction of the blood-sugar–raising factors lingers on as a conditioned reflex pattern; this explains the postoperative diabetes. Then, in a second adaptation process, the conditioned pattern gradually fades out and the carbohydrate tolerance returns to normal.

In the light of this interpretation, the deteriorated carbohydrate tolerance during the state of hyperinsulinism, as well as the transitory postoperative diabetes, represents a condition which was denoted a half century ago as *adrenalin diabetes*.

ARTIFICIAL HYPERINSULINISM

In years past, treatment with insulin injections was applied in various non-diabetic cases. Wilder et al. [*11*], for instance, turned to insulin injections in an attempt to reduce the body weight of obese individuals, while Clark et al. [*12*], Odin [*13*], Maher and Somogyi [*14*] and other workers tried to increase the body weight of anorexic persons by the same means. Such treatment, we find, entails disturbances in the carbohydrate metabolism which are virtually identical with those observed in organic hyperinsulinism: (1) the blood sugar shows diurnal fluctuations between extreme hypoglycemic and abnormally high hyperglycemic levels, occasionally accompanied by glycosuria; (2) glucose tolerance tests performed *during* the treatment give results characteristic of diabetes; and (3) after discontinuation of the injections, a transitory diabetes appears which persists for varying lengths of time.

Clark et al., who used up to 75 units of insulin daily, recorded postprandial (two hours after breakfast) blood sugar values ranging from 33 to 226 mg. per cent, occasional glycosurias during treatment, and post-treatment transitory diabetes lasting for several days. Glucose tolerance tests during and after treatment gave results similar to those obtained in diabetes. In one case transitory diabetes with glycosuria persisted for five days after discontinuation of the injections. Odin, who used up to 120 (3×40) units of insulin per day, observed a diabetic condition that persisted for over two weeks after stoppage of the injections. In one of his patients, who received only 16 (2×8) units per day, blood sugar values found during the day were as low as 20 to 40 mg. per cent, yet fasting sugar levels mounted as high as 160 to 170 mg. per cent. In general, Odin found that *the larger the insulin dosage and the longer the duration of the treatment, the greater was the diabetogenic effect of the treatment*. As the data given in Table II show, this is in line with the findings of Clark et al. and of Maher and Somogyi. The latter found progressive deterioration of the glucose tolerance when they used as little as 10 to 20 units of insulin over periods of many months. Odin's thesis is also borne out by Appel and Hughes [*15*], who treated mental patients with insulin shock therapy; in some of their

subjects transitory diabetes persisted for two or three months after the termination of insulin injections. Goia et al. [*16*], on the other hand, observed acute hyperglycemic-glycosuric response to insulin hypoglycemia after the injection of a single dose of insulin. These authors used insulin for treatment of bronchial asthma; the patients were injected with 50 units of insulin, and after the onset of the hypoglycemic reaction, usually two hours after the injection, they received a meal rich in carbohydrates. Two hours after the meal (four hours after injection) abnormal hyperglycemia was observed which persisted for another two to four hours, with mild degrees of glycosuria. Then postprandial glycosuria was observed for two or more days after the ending of the treatment.

We conclude from the foregoing facts that transitory diabetes of non-diabetic persons, as a response to insulin injections, is identical in kind with the response of patients suffering from organic hyperinsulinism; in other words, that the condition of non-diabetic persons treated with insulin represents a state of *artificial hyperinsulinism*. After all, it makes no difference whether the excess insulin is delivered from an islet cell tumor or from a syringe. The common denominator in the two conditions is excess insulin action, which produces observable, or even mild, asymptomatic, hypoglycemic periods; these in turn stimulate the adrenal-pituitary system to accelerated release of blood-sugar–raising hormones. Whenever the action of the latter outstrips insulin action, hyperglycemia and glycosuria make their appearance.

This interpretation is at sharp variance with the commonly adopted explanation of the transitory diabetes that is elicited by the treatment of non-diabetic persons with insulin. We deem the problem important enough to deal with it in a separate section.

DIMINISHED IRRITABILITY OF THE PANCREAS

The commonly accepted explanation of the phenomenon is that in persons in whom the endogenous supply of insulin is normal, the secretory activity of the islets subsides to a certain extent while they are receiving injected insulin. In other words, a state of hypoinsulinism is established which persists for variable periods of time after the injections are terminated. Wilder et al. expressed this view when they observed that in consequence of "administering

TABLE III

SHOWING PROGRESSIVE INCREASE IN HYPERGLYCEMIC AFTERMATH OF RECURRENT INSULIN HYPOGLYCEMIA

Breakfast Each Day at 7 A.M., Second Meal at 3 P.M.

Date	Insulin (units) (at 11 A.M.)	Mg. Glucose per 100 cc. Venous Blood					
		Noon	1 P.M.	2 P.M.	3 P.M.	4 P.M.	6 P.M.
9/17	35	48	27	..	..	33	132
9/18	40	19	26	36	37	62	167
9/20	40	25	23	24	56	63	188
9/21	45	30	19	10	27	57	238

of insulin for periods of from 12 to 30 days . . . glucose tolerance tests were obtained *typical of diabetes mellitus*," and assumed that: "The phenomenon *is explained by diminished irritability of the rested pancreas*." A few years later Clark et al. likewise stated: "The most *obvious explanation is that there is a compensatory inhibition of the normal islet secretion during the course of insulinization, and a slow readjustment* following the discontinuation of the insulin until normal activity is attained." (Italics in both quotations are ours.) This concept then was readily regarded by many authors as a self-evident fact. But there are several known facts which sharply militate against the assumption of a "diminished irritability of the rested pancreas" or a "compensatory inhibition of the normal islet secretion."

(1) In the first place, the assumption that islet activity is at rest during insulin injections is altogether unjustified. Insulin secretion undoubtedly is at low ebb when the blood sugar is at normal fasting or subnormal levels. This is the situation during every night and every postabsorptive interval. But it is not the case in subjects treated with insulin; in these the blood sugar intermittently rises to hyperglycemic peaks which are far above the normal postprandial levels, with the consequence that their islet tissues are day after day exposed to more aggressive stimulation than those of normal persons.

(2) Observations made in our laboratory on four mental patients treated with insulin show clearly the tenuousness of the dormant islets ("rested pancreas") theory. These patients received moderate doses of insulin (not larger than many diabetics are getting) and were exposed to hypoglycemic states for about four hours. When fed at the end of this period—as the example recorded in Table III shows—hyperglycemia and glycosuria developed during the third hour after the meal. As may be noted, the

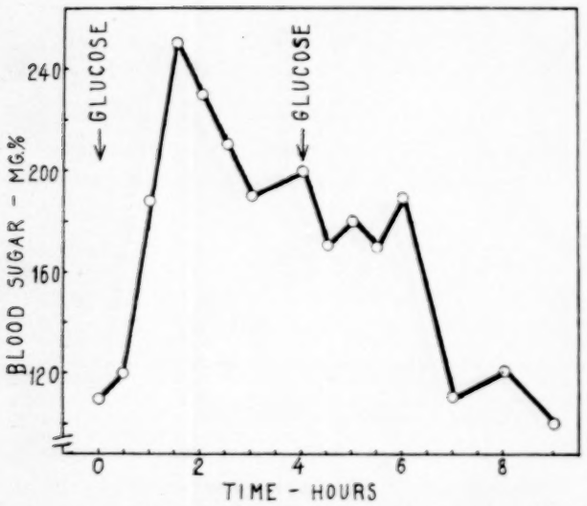

FIG. 1. Showing that diminished insulin action, in a rabbit that was starved for five days, was rapidly restored to normal under the stimulus of alimentary hyperglycemia, produced by administration of two 10 gm. doses of glucose.

diabetogenic effect of the insulin injections made its appearance on the second day of treatment and became quite obvious on the fifth day. Now, can this progressive deterioration of the carbohydrate tolerance be attributed to the dormancy of the pancreas? Not at all. As the data in Table III show, the islets of Langerhans were allowed to retreat into relative inactivity for only four-hour intervals, but not for the remaining twenty hours each day. Beyond this, the hyperglycemic states following in the wake of the hypoglycemic periods alerted the islets to a higher than normal rate of activity and thus prevented any cumulative effect of the brief rest periods. Therefore, we attribute the progressive deterioration of the carbohydrate tolerance in these cases to the cumulative diabetogenic effect of the repeated excitation of the adrenal-pituitary system by hypoglycemia.

The same phenomenon occurred in the experiments of Goldman [*17*], who treated non-diabetic mental patients with much larger doses of insulin. His purpose was to produce hypoglycemic reactions without severe shock, by keeping the patients on regular ward diet and giving them malted milk whenever needed to forestall severe shock. One of his patients (No. 24), who received 75 units of protamine-zinc insulin at twelve-hour intervals, i.e., 150 units per day, showed a blood sugar level of 30 mg. per cent at midnight, and eight hours later a hyperglycemia of 225 mg. per cent. Under the prevailing experimental conditions, far more extraneous insulin was available throughout all hours of the day than the amount required to confine the blood sugar within normal limits. After all, it is well known that totally pancreatectomized persons utilize adequate amounts of carbohydrates on 20 to 30 units of insulin [*18*] and can live on diets containing 400 gm. of carbohydrates with a single injection of 40 units of protamine-zinc insulin [*19*] or even 30 units [*20*] per day. Yet Goldman attributed the hyperglycemia to the subnormal activity of the pancreas, stating: "These findings may indicate inhibition of the patients' endogenous insulin secretion during the treatment."

(3) Rather weighty evidence contradicting this idea can be found in the half-century-old studies of Ivar Bang, who examined the glucose tolerance of rabbits after five days of starvation. In these tests two 10 gm. doses of glucose were fed to the rabbits four hours apart. The result of such an experiment is presented in the graph (Fig. 1), which was constructed from data given in Bang's monograph [*21*]. As may be seen, after the first dose of glucose the blood sugar shot up to diabetic-like levels, but after less than two hours the stimulus of hyperglycemia elicited the release of enough insulin to effect a fairly sharp decline. The action of an abundant insulin supply is even more accentuated after the administration of the second dose of glucose: here the blood sugar dropped instead of rising, showing the phenomenon which (incongruously) is denoted as the "Staub-Traugott effect." It is evident that islet activity was restored to a normal rate by the stimulus of hyperglycemia of a few hours' duration.

(4) Finally, experiments carried out in our laboratory [*22*] supply, we believe, added and conclusive proof to the effect that the abnormal hyperglycemic intervals in the state of artificial hyperinsulinism are triggered by hypoglycemia and not by hypofunctioning of the rested pancreas. In these experiments alimentary hyperglycemia, rising to peaks as high as in diabetes, was elicited in healthy subjects by a single injection of as little as 4 units of insulin. The hypoglycemia produced was of a mild, asymptomatic degree and was terminated forty minutes after the injection by the oral administration of glucose. As may be seen in Figure 2, the impact of such a slight excess of insulin action sufficed to raise the hyperglycemic peak by as much as

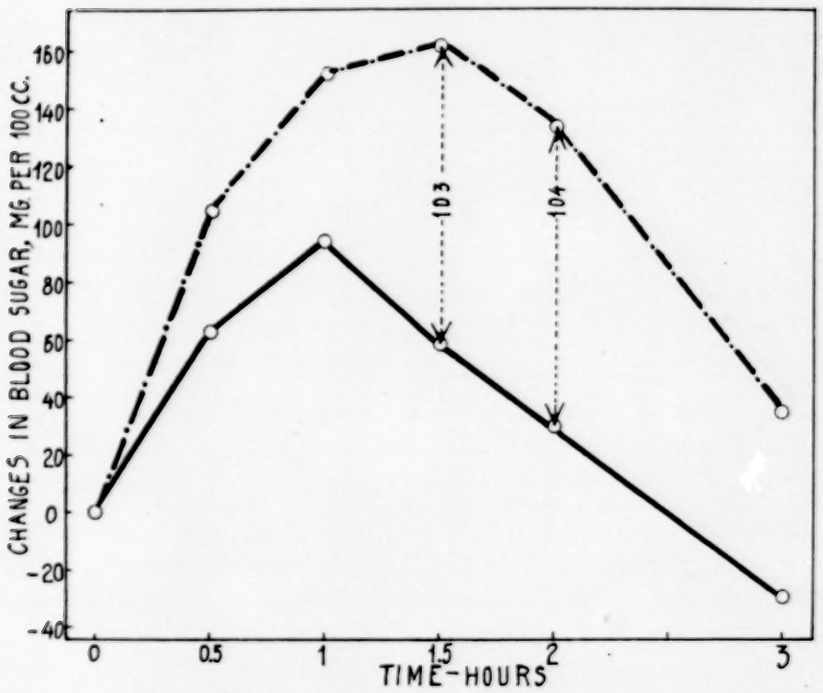

FIG. 2. Showing two glucose tolerance curves of a healthy person. ——— Conventional test with 100 gm. oral glucose. —·—·— 4 units insulin injected intravenously forty minutes before ingestion of glucose.

104 mg. per cent higher than it was when the ingestion of glucose was not preceded by artificial hyperinsulinism. The activity of the islets of Langerhans undoubtedly stayed at a low rate during the forty minutes of hypoglycemia; but it seems inconceivable that a brief rest period like this should have conditioned them to dormancy so deep that they could not be aroused to normal activity for several hours afterwards, even by abnormally high levels of hyperglycemia. Explanation of the impairment of the glucose tolerance on the basis of "inhibited islet secretion" or "diminished irritability" of the pancreas is made even more untenable in view of the fact that the greater part of the injected insulin was still present and active. It was working in addition to the endogenous insulin, which was released at an accelerated rate under the stimulus of hyperglycemia; but the action of this combined insulin supply was defeated and outstripped by its opponents, which were alarmed by hypoglycemia to excessive activity.

This interpretation of the diabetogenic effect of artificial hyperinsulinism was an upshot of our observations on patients with severe, unstable diabetes. Persuaded that in such cases excess insulin action accounts for the deterioration of the carbohydrate tolerance, we could not doubt that it exerts the same effect in non-diabetic persons. Thus, in 1938, ignoring the dominant but unsubstantiated theory, we interpreted the transitory diabetes of non-diabetic persons treated with insulin as follows [*14*]: "This phenomenon, in our opinion, is closely related to that observed on diabetic patients who are treated with excessive doses of insulin. In the case of the diabetic any amount of insulin that depresses the blood sugar below the fasting level represents an overdose which entails high degrees of hyperglycemia, i.e., a further diminution of carbohydrate tolerance. In the case of the nondiabetic, whose pancreas supplies adequate amounts of insulin, any added quantity of extraneous insulin represents an excess and leads to the deterioration of carbohydrate tolerance just as overdosage does in the diabetic."

This early, pragmatic comment is amplified and validated by identification of the neuroendocrine reactions which account satisfactorily for the diabetogenic aftermath of insulin hypoglycemia.

SUMMARY

1. An interpretation is submitted concerning the paradoxical deterioration of the carbohy-

drate tolerance in persons who, owing to islet cell tumors of the pancreas, suffer from organic (endogenous) hyperinsulinism. The explanation offered is that hypoglycemia, produced by excess insulin action, induces an accelerated release of blood-sugar–raising hormones from the adrenal-pituitary system to an extent that under certain conditions the action of the excited insulin-opposing factors outstrips insulin action. Hence, the diabetic-like response to glucose tolerance tests during the state of hyperinsulinism, as well as the transitory diabetes after surgical elimination of the excess insulin supply, are manifestations of "adrenalin diabetes."

2. Artificial hyperinsulinism, produced by giving insulin injections to non-diabetic persons, is accompanied by much the same abnormalities as organic hyperinsulinism, since it activates the same diabetogenic factors. Insulin treatment which is allowed to cause hypoglycemia in diabetic patients places them in a *de facto* state of artificial hyperinsulinism, with the consequence that adrenalin diabetes is superimposed on an already deranged state of metabolism. Evidence is presented showing that the greater the excess of insulin and the longer a subject is exposed to its adverse effect, the greater is the diabetogenic effect of the treatment.

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Coagulation Defects in Liver Disease and Response to Transfusion During Surgery*

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HEMORRHAGE is a well recognized and serious hazard in patients with severe liver disease, particularly during and following surgery. The opportunity to study this problem has been presented by the increasing number of patients with cirrhosis, portal hypertension and bleeding esophageal varices subjected to splenorenal or portacaval shunt surgery. The use of fresh, whole blood transfusion has been reported to be associated with a marked decrease in mortality from hemorrhage in this group of patients [*1*]. In the present study we have attempted to determine the nature and degree of the coagulation defects of liver disease and the response of these defects to transfusion during surgery.

The Coagulation Mechanism. For practical clinical purposes coagulation may be considered as occurring in three phases: phase I, evolution of thromboplastin; phase II, conversion of prothrombin to thrombin by thromboplastin; phase III, conversion of fibrinogen to fibrin by thrombin. The known coagulation factors and inhibitors which play a role in each of these phases of coagulation are listed in Table I.

The thromboplastin generated in phase I, which may be referred to as plasma thromboplastin or total thromboplastin, can directly convert prothrombin to thrombin in the presence of calcium. The thromboplastin generation test is a screening test for factors involved in phase I. If tissue thromboplastin (saline extract of lung or acetone-dried brain), which is a partial thromboplastin, is substituted for plasma thromboplastin, certain plasma factors in addition to prothrombin are essential for thrombin forma-

TABLE I

Coagulation Factors	Coagulation Inhibitors
Phase I (Evolution of Thromboplastin)	
Ca^{++}	
Platelets	
Antihemophilic factor (AHF, AHG, AHFA)	Anti-AHF
Nishimine factor ?	
Plasma thromboplastin component (PTC, Christmas factor, factor IX, AHFB)	Anti-PTC
Plasma thromboplastin antecedent (PTA)	Anti-PTA
Hageman factor	
Car factor ?	
Stuart factor (Prower factor)	
Accelerator globulin (Ac-globulin, labile factor, factor V, proaccelerin)	Antiaccelerator globulin
Factor X	
Phase II (Conversion of Prothrombin to Thrombin)	
A. *Plasma* thromboplastin plus	Antiplasma thromboplastin
Ca^{++}	
Prothrombin	
B. *Tissue* thromboplastin plus	Anti-tissue thromboplastin
Ca^{++}	
Accelerator globulin (factor V, labile factor, proaccelerin)	Antiaccelerator globulin
Proconvertin (pro-SPCA, factor VII, stable factor, cothromboplastin)	Antiproconvertin
Stuart factor (Prower factor)	
Factor X	
Prothrombin	
Phase III (Conversion of Fibrinogen to Fibrin)	
Thrombin	Anti-thrombin
Fibrinogen	
Fibrin	
Fibrin + platelets + serum factor → clot retraction	

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tion, namely proconvertin, accelerator globulin, Stuart factor and possibly factor X. The other factors listed in phase 1 are not essential. The screening test for the factors involved in this modified second phase reaction is the Quick prothrombin time. (Fibrinogen is also essential for the end point in the Quick test, but this factor does not play a role in prothrombin conversion.) In the third phase of coagulation, fibrinogen is converted to fibrin by thrombin. The thrombin is neutralized by antithrombin. However, this reaction proceeds at a slower rate than the conversion of fibrinogen to fibrin by thrombin, so that normally it does not interfere with the latter process. Clot retraction results from an interaction between fibrin, platelets and a serum factor. Fibrinolysis results from the activation of an inert precursor in plasma (profibrinolysin or plasminogen) to the active form, fibrinolysin or plasmin. This enzyme degrades both fibrinogen and fibrin, the latter more rapidly. Plasmin is neutralized by another factor in plasma, antiplasmin.

METHODS AND MATERIALS

Blood was obtained using untreated syringes and needles; the first syringe was discarded. When plasma was required, 9 volumes of blood were mixed with 1 volume of anticoagulant.* The cells were sedimented at 2,000 revolutions per minute for twenty minutes in glass tubes.

Veronal® buffered saline solution (VBIS) was prepared by adding 200 ml. of 0.1 M sodium barbital to 144 ml. of 0.1 N HCl and adding 0.9 per cent sodium chloride solution to bring the final volume to 1,000 ml. The pH was 7.4.

First Phase of Coagulation. 1. Thromboplastin generation was tested by the method of Biggs and Douglas [2] with the following modifications: (A) A partial thromboplastin, asolectin,† (20 mg./100 ml.) was substituted for a suspension of washed platelets. (B) Adsorbed plasma was prepared from oxalated rather than citrated plasma, and barium sulfate (100 mg./ml. of plasma) was substituted for aluminum hydroxide as the adsorbing agent. (C) After clotting, the serum was kept at room temperature for twelve to twenty-four hours before use. (D) Veronal buffered saline solution (VBIS) was used to make all dilutions. When thromboplastin generation was abnormal, attempts at correction were made by substitution of normal barium sulfated plasma or normal serum for the patient's plasma or serum. By this means it could be determined whether the defect was in the plasma, in the serum or in both.

Recent studies have revealed that in addition to PTC, the Stuart factor [3], and perhaps factor X [4] and the Car factor [5], present in serum but not in barium sulfated plasma, are also essential for normal thromboplastin generation. Therefore serum abnormalities as determined by the thromboplastin generation test may be secondary to deficiencies of one or more of these factors. For this reason the term "serum thromboplastic activity" will be used to refer to the factors supplied only by the serum in the thromboplastin generation test. Similarly, both AHF and Ac-globulin, and perhaps other factors (Nishimine [6]) present in $BaSO_4$ plasma but not in serum, are essential for normal thromboplastin generation. The term "plasma thromboplastic activity" will therefore be used to refer to the factors supplied only by the plasma in the thromboplastin generation test. PTA and Hageman factor are present in both serum and $BaSO_4$ plasma; a deficiency of either or both of these factors is corrected by the incorporation of either normal serum or normal adsorbed plasma in the generation test.

The thromboplastin generation test was used as a semi-quantitative measure of the serum and plasma thromboplastic activities. Fresh serum was taken from six normal donors, pooled and diluted 1:10 with Veronal buffered saline. The pooled diluted serum was designated as containing 100 per cent serum thromboplastic activity. Further dilution was made with a 1:10 dilution of barium sulfate adsorbed normal serum in VBIS (thus maintaining plasma thromboplastin antecedent and Hageman factors relatively constant) to obtain concentrations of 50, 30, 15 and 5 per cent serum thromboplastic activity, respectively. Thromboplastin generation tests were then carried out in the usual manner with each of the five dilutions of serum. The curves obtained by plotting the clotting time against incubation time represented the generation of thromboplastin from known concentrations of serum thromboplastic activity and were used as a basis for estimating this activity in unknown samples. A patient's serum thromboplastic activity was determined by comparing the thromboplastin generation curve (obtained by using a 1:5 dilution of normal barium sulfated plasma and a 1:10 dilution of the patient's serum) with the thromboplastin generation of the varying dilutions of normal serum. Abnormalities of serum thromboplastic activity were recorded as 1 plus (50–30 per cent), 2 plus (30–15 per cent), 3 plus (15–5 per cent), and 4 plus (<5 per cent of normal). (Fig. 1.) Plasma thromboplastic activity was determined in a similar manner using varying dilutions of $BaSO_4$-adsorbed normal plasma in a 1:10 dilution of $BaSO_4$-adsorbed hemophilic plasma to prepare the reference curves.

* Sodium oxalate, 0.1 M, was used to obtain plasma for the thromboplastin generation test, and 2.5 per cent sodium citrate to obtain plasma for prothrombin, proconvertin and Ac-globulin tests.

† Supplied by the Associated Concentrates Company, Woodside, New York.

2. Prothrombin consumption was determined by the method of Alexander [7].

3. Platelets were counted by the direct Rees and Ecker method [8] (N = 200,000 to 450,000 per cu. mm.).

Second Phase of Coagulation. Prothrombin times were determined by the Quick one-stage method [9] and prothrombic activity was determined from a dilution curve of normal plasma using normal $BaSO_4$-adsorbed plasma as the diluent. Accelerator globulin was determined by the method of Alexander [10] and proconvertin and prothrombin concentrations by a modification of Owren's method [7]. Simplastin®* was used as the tissue thromboplastin in all these procedures.

Third Phase of Coagulation. Fibrinogen was determined quantitatively by the method of Cullen and Van Slyke [11]. Citrated plasma (0.5 ml.) was diluted with 15 ml. physiologic saline solution, recalcified, the fibrin separated, washed, and its nitrogen content determined by digestion and nesslerization. (If the Prothrombin time was prolonged, thrombin (100 units) was added in place of calcium.) Fibrinolytic activity was estimated by inspection of the whole blood clot after one, two, four, twelve and twenty-four hours of incubation at 37.5°c. Complete dissolution of the clot within the first twenty-four hours was considered as increased fibrinolysis. (Incomplete dissolution of the clot within the period of observation was not considered fibrinolysis, because normal syneresis of the clot with sedimentation of some red cells could not be distinguished from incomplete lysis.)

Vascular Phase. Bleeding times were determined in a small number of patients by Ivy's technic as modified by Jacobson [12]. Capillary fragility was performed in a few cases with a specially designed suction petechiometer, maintaining negative pressures of 250 mm. Hg for one minute. The normal response was less than 10 petechiae in a circle 3 cm. in diameter.

Other Tests. Clotting times of whole blood were determined at 37.5°c. in three 12 by 100 ml. serologic test tubes by a modification of the method of Lee and White [13]. Clot retraction was evaluated by gross inspection of the clot at one and four hours.

SELECTION OF PATIENTS AND PLAN OF STUDY

Twenty-five hospitalized patients were selected for study. There were ten male patients and fifteen female patients; all were Caucasian. The mean age was forty-five years, with an age range from ten to seventy-nine. There were twelve patients with Laennec's cirrhosis, seven with postnecrotic cirrhosis, two with acute viral hepatitis (one died of acute yellow atrophy), one with obstructive jaundice and three with extrahepatic portal hypertension. The diagnosis was made by autopsy or biopsy of the liver in every case except one, a patient (Case II) with acute viral hepatitis.

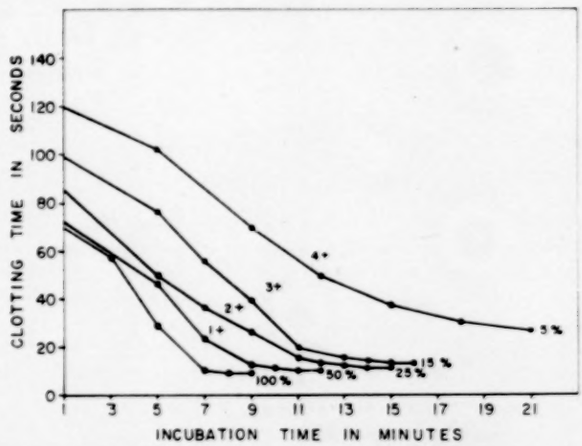

FIG. 1. Dilution curve for serum thromboplastic factors. These curves represent the thromboplastin generating activity of serial dilutions of normal serum and were used to determine the degree of abnormality of serum thromboplastic factors in the patient.

Baseline coagulation studies were performed in all patients. Nineteen of these patients were also studied during and after surgery. Sixteen of the latter group had hepatic disease and three had extrahepatic portal hypertension. There were nine splenorenal shunt operations (with splenectomy), three end-to-side portacaval shunts, one coronary vein–inferior vena cava anastomosis, one transthoracic ligation of bleeding esophageal varices, one splenectomy, three subtotal gastrectomies and one Tanner procedure.

The number of transfusions varied from 1 to 8 units (500 to 4,000 ml.) with an average of between 3 to 4 units.* As these studies were a corollary of the surgical procedures, no attempt was made to control the rate of blood replacement or age of the blood administered.† In each case the banked blood collected in bottles had been stored eleven to eighteen days. Fresh blood collected in bottles and stored less than twenty-four hours was given to every patient receiving more than 3 units of stored bank blood (1,500 ml.). The proportion of fresh to stored blood was in most instances approximately 1:2. In seven patients coagulation studies were repeated the day following operation and in ten cases three to twenty-one days postoperatively. Splenectomy was performed in ten of these nineteen patients and in one patient (Case XVII) not studied during surgery. In these eleven cases platelet counts were performed at frequent intervals during the postoperative period (one to thirty-two days).

* Available from Warner-Chilcott Laboratories, Morris Plains, New Jersey.

* All blood administered was collected in acid citrate dextrose solution, A.C.D. (approved National Institute Health formula A: acid citrate 0.8 gm.; dextrose 2.45 gm.; and trisodium citrate, 2.2 gm. per 100 ml. of water; 120 ml. per unit).

† In two cases fresh blood was employed exclusively.

TABLE II

Patient, Sex and Age (yr.)	Diagnosis	Liver Function Studies					Coagulation Studies				
		Serum Bilirubin (mg. %)	Bromsulphalein Retention (%)	Cephalin Flocculation Test	Serum Albumin (gm. %)	Serum Globulin (gm. %)	Clotting Time (15 min. Upper Limit) (min.)	Clot Retraction	Platelets (cu. mm.)	Bleeding Time (min.)	Capillary Fragility
I, M, 79	Viral hepatitis (acute yellow atrophy)	39.2	..	4+	2.6	4.9	23	Fair	43,000	..	.....
II, F, 22	Acute viral hepatitis (mild)	3.7	..	4+	...	...	13	Fair	172,000	..	.....
*III, M, 41	Laennec's cirrhosis	3.6	..	4+	2.7	3.2	17	Fair	97,000	..	.....
*IV, F, 50	Laennec's cirrhosis	.8	..	3+	4.6	3.2	15	Good	172,000	3	Normal
V, M, 43	Laennec's cirrhosis	14.4	..	...	2.4	5.1	14	Good	95,000	..	.....
*VI, F, 55	Laennec's cirrhosis	3.0	38	3+	2.6	4.0	13	Good	122,000	..	.....
*VII, M, 51	Laennec's cirrhosis	5.6	..	...	...	4.6	13	Good	98,000	..	.....
*VIII, F, 34	Laennec's cirrhosis	1.1	15	4+	4.3	3.7	14	Good	86,000	..	.....
*IX, F, 68	Laennec's cirrhosis	.4	16	3+	3.7	4.5	12	....	57,000	..	Normal
*X, F, 41	Laennec's cirrhosis	2.0	23	4+	2.9	3.0	11	Good	69,000	..	.....
XI, M, 50	Laennec's cirrhosis	5.8	..	4+	2.5	4.6	12	Good	73,000	..	.....
*XII, F, 39	Laennec's cirrhosis	1.6	..	...	2.5	2.5	12	Good	148,000	..	Normal
*XIIa, F, 39	Same patient as above, terminal	8.5	..	...	3.7	1.4	10	Fair	40,000	..	.....
*XIII, F, 56	Laennec's cirrhosis	3.0	..	4+	2.6	3.5	6	....	.....	..	.....
†XIV, F, 37	Laennec's cirrhosis	1.5	..	2+	5.0	3.4	23	Good	104,000	..	Abnormal
†XV, M, 43	Postnecrotic cirrhosis	0.8	23	3+	3.3	3.4	17	....	70,000	..	Abnormal
*XVI, M, 60	Postnecrotic cirrhosis	6.6	43	4+	...	3.6	16	Good	56,000	..	.....
‡XVII, M, 10	Postnecrotic cirrhosis	3.0	32	3+	2.4	2.6	13	Fair	42,000	12	Abnormal
*XVIII, F, 51	Postnecrotic cirrhosis	5.2	46	4+	2.9	3.1	12	Good	91,000	..	Normal
*XIX, F, 54	Postnecrotic cirrhosis	....	9	2+	3.7	4.0	10	Poor	67,000	3	.....
*XX, M, 29	Postnecrotic cirrhosis	1.4	..	2+	3.1	1.9	14	Poor	196,000	..	.....
*XXI, F, 37	Postnecrotic cirrhosis	0.8	11	2+	4.0	2.2	15	Good	126,000	2	Normal
XXII, M, 53	Obstructive jaundice (carcinoma of pancreas)	32.1	..	0	3.4	2.8	34	Good	397,000	..	.....
XXIII, F, 47	Extrahepatic portal hypertension	....	2	1+	4.2	1.7	11	Good	103,000	3	.....
*XVIV, F, 41	Extrahepatic portal hypertension	0.5	1	0	4.2	2.6	17	Good	306,000	9	Abnormal
*XXV, F, 13	Extrahepatic portal hypertension	1.7	4	2+	4.4	2.3	15	Good	233,000	..	.....

* Patients followed during surgery.
† All fresh blood administered during surgery.
‡ Postsplenectomy platelet response studied but not followed during surgery.

RESULTS

Preoperative and Pretransfusion Coagulation Studies. (Table II.) *Parenchymatous hepatic disease* (twenty-one patients): In diffuse liver disease multiple coagulation defects involving all phases of coagulation were usually observed. Of sixteen patients tested, nine showed a moderate abnormality of serum thromboplastic activity, while in two patients (Case I acute yellow atrophy, and Case XII terminal cirrhosis) this abnormality was as severe as that encountered in hereditary PTC deficiency (Christmas disease). In one of these patients and in another patient with acute yellow atrophy not included in this study a specific assay for PTC was made and found to be markedly depressed. (Stuart factor and factor X were not measured.) Slight plasma thromboplastic abnormalities were present in four patients. Marked accelerator globulin deficiency was present in all these four patients (11, 18, 16 and 34 per cent). The plasmas of two of these patients (Case I 11 per cent Ac-globulin, and Case III 34 per cent Ac-globulin) on further analysis were found to have normal titers of AHF when measured by their corrective effect upon the prothrombin consumption of the blood of a patient with hemophilia A (AHF deficiency). In the experience of one of us (R. G.) and others [*14*] the thromboplastin generation test is affected when accelerator globulin falls below 30 to 35 per cent, and it is probable that the

TABLE II (*Continued*)

Special Studies			Coagulation Studies						
Thromboplastin Generation	Plasma Thromboplastin Factors	Serum Thromboplastin Factors	Prothrombin Consumption (Normal Value Over 85%) (%)	Quick Prothrombin Concentration (Normal Value 70 to 120%) (%)	Owren Prothrombin Concentration (Normal Value 70 to 120%) (%)	Accelerator Globulin (Normal Value 70 to 120%) (%)	Proconvertin (Normal Value 70 to 120%) (%)	Fibrinogen (gm. %)	Fibrinolysis (Normal Value 0 in 24 hr.)
Abnormal	Slightly abnormal	4+	80	5	5	11	6	...	0
Abnormal	Normal	2+	..	70	55	100	64	290	0
Abnormal	Slightly abnormal	1+	87	41	40	34	39	90	0
Abnormal	Normal	1+	..	59	66	42	58	330	0
Normal	Normal	Normal	97	16	45	33	31	190	0
.....	.....	.....	85	..	68	68	57	250	0
Abnormal	Normal	1+	98	22	54	31	39	350	Complete in 24 hr.
.....	.....	.....	86	63	55	57	53	300	0
Normal	Normal	Normal	..	67	62	61	78	350	Complete in 2 hr.
Abnormal	.....	.....	75	20	62	24	58	170	0
Abnormal	Slightly abnormal	2+	94	9	18	18	24	160	0
Normal	Normal	Normal	..	48	68	62	60	360	0
Abnormal	Slightly abnormal	3+	99	12	12	16	8	80	Complete in 12 hr.
.....	.....	.....	..	..	39	15	22	280	0
Abnormal	Normal	2+	..	44	58	51	68	...	0
Abnormal	Normal	1+	..	33	40	42	45	290	Complete in 24 hr.
Abnormal	Normal	2+	97	23	47	35	39	430	0
Normal	.....	.....	87	43	80	70	64	220	0
Abnormal	Normal	1+	..	26	44	36	52	280	0
Normal	Normal	Normal	98	60	66	76	58	600	0
.....	.....	.....	81	79	74	74	62	280	Complete in 4 hr.
Normal	Normal	Normal	98	28	82	49	56	300	0
Abnormal	Normal	3+	94	2	19	94	6	350	0
Normal	Normal	Normal	94	42	94	60	74	220	0
Normal	Normal	Normal	98	47	98	80	88	740	0
Normal	Normal	Normal	95	81	74	115	75	440	0

mild decrease in plasma thromboplastic activity in these four cases was due to a deficiency of accelerator globulin and not of antihemophilic factor, a conclusion in accord with previous reports [*15,16*]. Although plasma thromboplastin antecedent (PTA) and Hageman factor levels were not specifically determined, the results of thromboplastin generation tests were not suggestive of a deficiency in either of these factors. These findings are not in agreement with Naeye's report [*17*] that PTA deficiency is a common finding in patients with liver disease.

Every patient with parenchymatous hepatic disease had slight to severe thrombocytopenia. Most of the patients had advanced cirrhosis complicated by congestive splenomegaly and portal hypertension. The platelet depression may be severe enough to be a major factor in the hemorrhagic tendency in advanced disease of the liver.

In agreement with other reports [*15,16,18–20*], the most pronounced changes were encountered in the factors involved in the second phase of coagulation as measured with tissue thromboplastin. Prothrombin, accelerator globulin and proconvertin were depressed. The severest abnormalities were seen in a patient with acute yellow atrophy of the liver (Case 1 prothrombin 5 per cent, Ac-globulin 11 per cent and proconvertin 6 per cent). In general these factors were depressed in parallel, with the exception of a single patient with acute viral hepatitis (Case

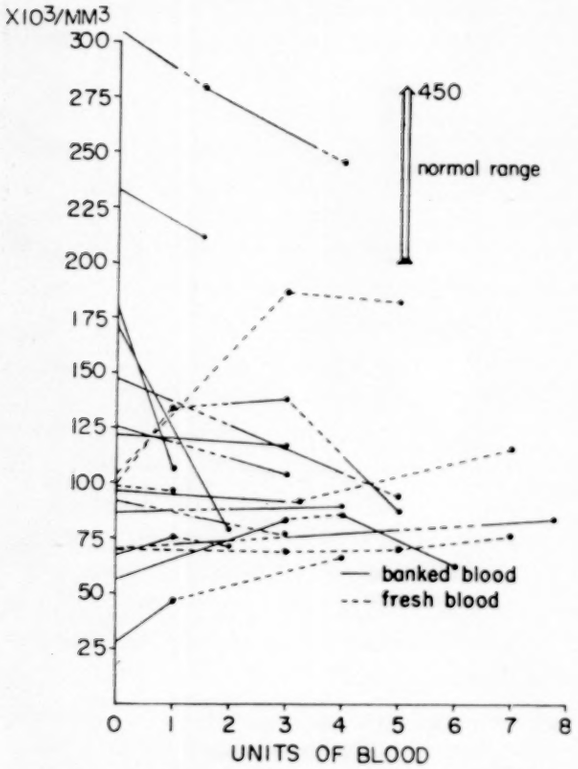

FIG. 2. Effect of serial blood transfusions on platelet count in patients with liver disease. The two patients with platelet levels in the normal range preoperatively suffered from extrahepatic portal obstruction with essentially normal liver function.

II) in whom accelerator globulin was normal but there was a moderate deficiency of prothrombin and proconvertin.

Fibrinogen was below 100 mg. per cent in two patients, but more commonly was normal or only slightly lowered. Fibrinolysis was observed in 20 per cent of patients with parenchymal-hepatic disease.

The severity of the coagulation disturbance roughly paralleled the derangement of liver function as assessed by the usual liver function studies. Pronounced depression of coagulation factors occurred when the cephalin flocculation test was 3 plus or 4 plus, serum albumin less than 3 gm. per cent, serum bilirubin greater than 1 mg. per cent or the Bromsulphalein® retention greater than 15 per cent.

Obstructive jaundice (Case XXII): The coagulation studies of one case of severe obstructive jaundice are included to illustrate the characteristic difference between the findings in obstructive and parenchymatous disease. In both conditions prothrombin, proconvertin and serum thromboplastic activity are depressed; however, in obstruction the accelerator globulin is normal or elevated [*18,20*] whereas in parenchymatous disease, except in early mild to moderately severe viral hepatitis (Case II), it is depressed, usually in proportion to the depression of the proconvertin and prothrombin. In addition, in obstruction jaundice the platelet and the fibrinogen levels are usually normal. The parenteral administration of vitamin K corrected the coagulation defect in this patient within twenty-four hours, which is characteristic of the defects seen in obstructive jaundice. Vitamin K has no effect or only a slight transitory effect upon the coagulation defect in parenchymatous liver disease.

Extrahepatic portal hypertension with normal liver function (three patients): In contrast to the patients with hepatic disease, the coagulation studies were normal in these patients except for mild thrombocytopenia in one case.

Effects of Blood Transfusion on Coagulation Factors during Surgery. Platelets: Of nineteen patients studied during surgery and transfusion only three had normal platelet values preoperatively (two with extrahepatic portal hypertension and one with cirrhosis). (Fig. 2.) Both of the patients with extrahepatic portal hypertension and normal platelet levels exhibited a fall in platelets but not to thrombocytopenic levels. The patient with liver disease (not included in Figure 2) showed no change. Of the sixteen patients with definite thrombocytopenia, further platelet depression coincident with transfusion and surgery developed in three, there was essentially no alteration of the platelet level in nine, there was a steady rise in platelets in two, and there was a transient rise in two. Since most of the patients received both bank and fresh* blood, it is difficult to correlate the change in platelet levels with the age of the blood administered. However, one patient given all fresh blood, 7 units of seventeen to twenty-one hour old blood, failed to show an increase in platelets (Fig. 3) whereas another patient who received 5 units of one to four hour old blood demonstrated a rise from 104,000 to 185,000 per cu. mm. (Fig. 8.) Although removal of the spleen may have contributed to the rise in platelets in this case it is more likely that the

* Bank = eleven to eighteen days old, with an average platelet count of 87,000/cu. mm. (range 56,000 to 109,000/cu. mm.). Fresh = zero to twenty-four hours old, with an average platelet count of 221,000/cu. mm. (range 146,000 to 493,000/cu. mm.) [*32*].

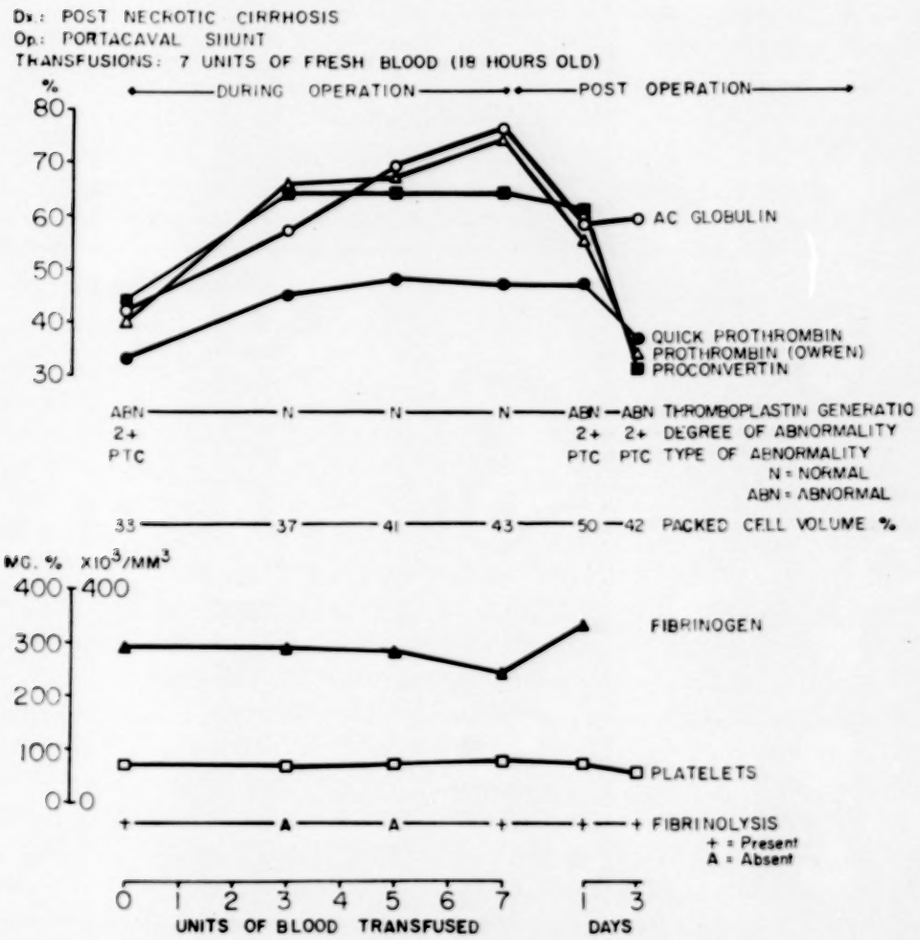

FIG. 3. Effect of transfusions of eighteen hour old blood on coagulation factors during surgery. Significant increases in concentrations of prothrombin, proconvertin and Ac-globulin and improvement in thromboplastin generation occurred. There was transient correction of fibrinolysis. No change in platelets occurred. By the third postoperative day concentrations of coagulation factors had returned to their previous low levels.

blood was responsible since significant increases in platelet counts during surgery were not observed in nine other patients with parenchymatous liver disease who underwent splenectomy. In five of these operations fresh blood (one to four hours old) was used in combination with stored bank blood and no significant change in platelet levels occurred. Thrombocytopenia following multiple transfusions of bank blood has been demonstrated by others [*21,22*] and confirmed by us. Marked platelet depression usually occurs only after massive transfusions (5,000 ml. or more in adults) although moderate falls in platelet levels have been noted not infrequently after administration of 2,000 to 3,000 ml. of bank blood. None of our patients needed massive transfusions and it should be noted that all patients receiving more than 1,000 ml. of blood were given fresh blood (one to twenty-four hours old) on an average of 1 unit of fresh blood for every 2 units of bank blood. Prior to the introduction of fresh blood transfusions (1949), when severe operative hemorrhage in these patients was often observed [*1*], many patients required blood in excess of 4,000 ml. during surgery, and it is quite possible that platelet depression secondary to transfusions may have been in part responsible for the severe hemorrhage. From published reports [*21,22*] it would appear that most platelets remain viable for about four hours after blood is drawn either into bottles or plastic bags. After four hours of storage there is a steady decline in viability even in the blood in plastic bags, so that at the end of twenty-four to forty-eight hours of storage few if any platelets survive *in vivo* [*22*].

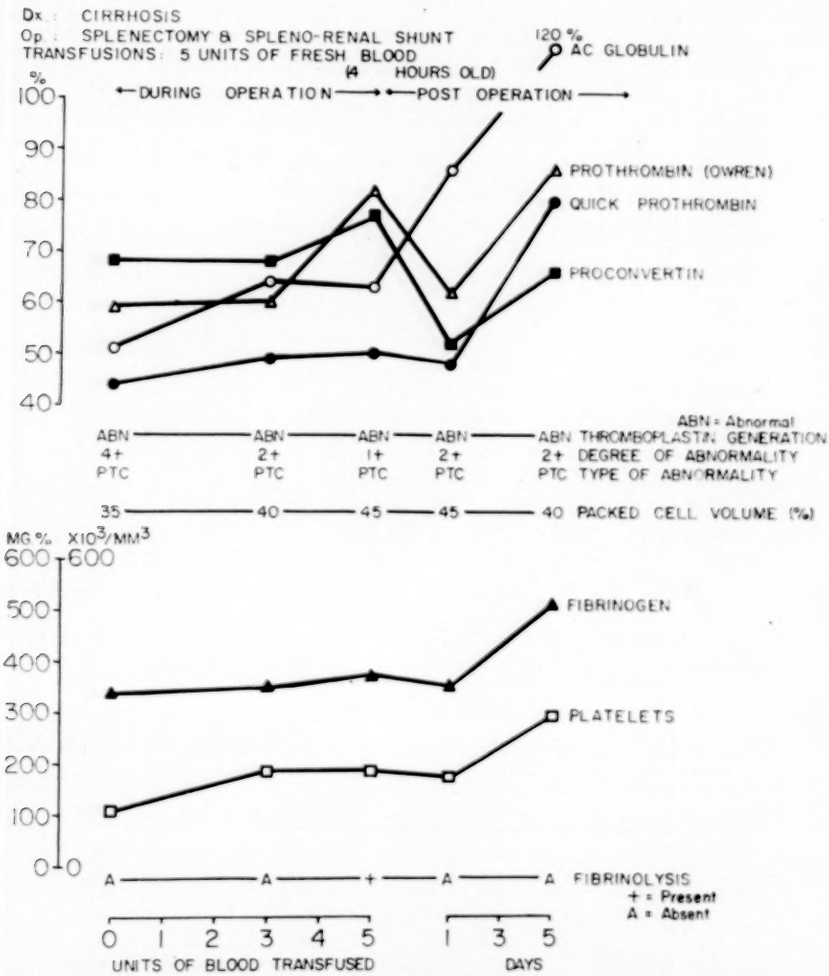

FIG. 4. Effect of transfusions of four-hour old blood on coagulation factors during surgery. Note moderate rise in second phase coagulation factors. Progressively beneficial effect on thromboplastin generation during transfusion; note the appearance of fibrinolysis at end of surgery. Note also rise in platelets from 104,000 to 185,000/cu. mm. Beneficial effects of transfusion had disappeared on the first postoperative day, but subsequently there was marked improvement in coagulation, unusual in this group of patients.

Serum thromboplastic activity (PTC, etc.): Serum thromboplastic deficiencies which were present preoperatively in six patients were corrected to normal in every instance after 1 to 3 units of blood; both bank and fresh blood were apparently equally effective. (Figs. 3 and 4.) There was no deterioration of serum thromboplastic activity in the seven patients with normal preoperative levels.

Plasma thromboplastic activity (AHF, Ac-globulin): Plasma thromboplastic activity appeared to be unaffected by transfusion as evaluated by the thromboplastin generation test.

Accelerator globulin: The response of accelerator globulin to transfusion is illustrated in Figure 5. Although there was considerable individual variation, an over-all pattern is apparent. When accelerator globulin was above 60 per cent, there was a tendency for this factor to fall slightly, whereas if below 40 per cent initially the tendency was to rise. The effect of transfusion on accelerator globulin was dependent not only on the amount but also on the age of the blood given. It has been shown by Mustard [*14*] and by others that the accelerator globulin content of bank blood deteriorates with time. In eleven to eighteen day old A. C. D. blood in the Massachusetts General Hospital blood bank the average accelerator globulin content was 25 per cent of normal with a range of 18 to 35 per cent,

whereas blood twenty-four hours old or less had 90 per cent activity. As would be expected, the administration of large amounts of stored blood may result in a significant decrease in accelerator globulin. For example, in one case with an initial level of 62 per cent accelerator globulin, the administration of 6 units of blood (1 unit of fresh blood) resulted in a fall to 35 per cent. (Fig. 6.) In contrast, the administration of 7 units of fresh blood (eighteen hours old) to a patient with an initial level of 40 per cent resulted in a rise to 75 per cent. (Fig. 3.) All six patients with initial levels of 40 per cent or less improved as the result of transfusion, employing both fresh and stored blood. The greatest rise in this factor using combined bank and fresh blood transfusion was from 34 to 53 per cent following 7 units (4 units of fresh blood). (Fig. 5.) Accelerator globulin was the single plasma factor in which a clear-cut superiority of fresh blood over routinely banked blood was observed.

Proconvertin: The changes in proconvertin following transfusion are shown in Figure 7. In general the changes in this factor were not pronounced. If low initially, improvement usually followed; if normal, slight depression developed. Marked depression of proconvertin, in contrast to accelerator globulin, did not follow transfusion of large amounts of stored bank blood, and in this regard the superiority of fresh blood over bank blood was not apparent. One patient with an initial level of 78 per cent showed no change after receiving 6 units of blood, only 1 of which was fresh. (Fig. 6.) This might be anticipated in view of the normal concentration of proconvertin in eleven to eighteen day old blood (average 82 per cent with a range from 43 to 115 per cent). In the patient receiving 7 units of fresh blood (eighteen hours old) the proconvertin increased from 45 to 65 per cent. (Fig. 3.)

Prothrombin concentration: Prothrombin changes were similar to proconvertin. (Fig. 8.) Profound depression of prothrombin as a result of blood replacement did not occur. Again this might be predicted in view of the near normal concentration of prothrombin in stored A. C. D. blood (average 72 per cent with a range of 66 to 78 per cent in eleven to eighteen day old A. C. D. blood). The greatest rise in prothrombin concentration followed 7 fresh blood transfusions (40 to 75 per cent) (Fig. 3) but in general stored blood appeared to be as effective as fresh blood in maintaining or correcting this factor.

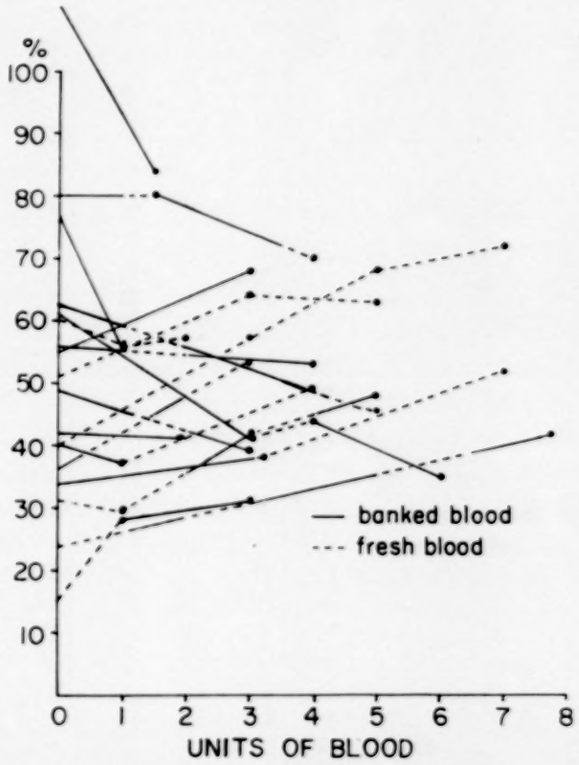

FIG. 5. Effect of serial blood transfusions on accelerator globulin in patients with liver disease.

Fibrinogen: The response of fibrinogen to transfusion (Fig. 9) was erratic and of slight degree in all but two instances. In one patient (Case VII), with severe cirrhosis who was undergoing splenectomy and splenorenal shunt, hypofibrinogenemia developed, accompanied by complete blood clot lysis in two hours. The fibrinogen concentration fell from 350 to 50 mg. per cent in spite of 5 units of blood (3 units of fresh blood). Abnormal bleeding was noted during and following surgery (to be discussed more fully subsequently). The other patient (Case III) demonstrated a rise in fibrinogen from 90 to 240 mg. per cent following 7 blood transfusions (4 units of fresh blood). The fresh blood did not cause any greater rise than the bank blood.

Fibrinolysis during Surgery. Observations on fibrinolysis were made in thirteen patients subjected to surgery. During or after surgery complete lysis of the clot in less than twenty-four hours was present in five cases. In two of the five an abnormal degree of fibrinolysis was present prior to surgery. In one of these (Case VII), however, the rate of lysis during surgery increased from complete lysis in twenty-four hours to complete lysis in two hours, and in

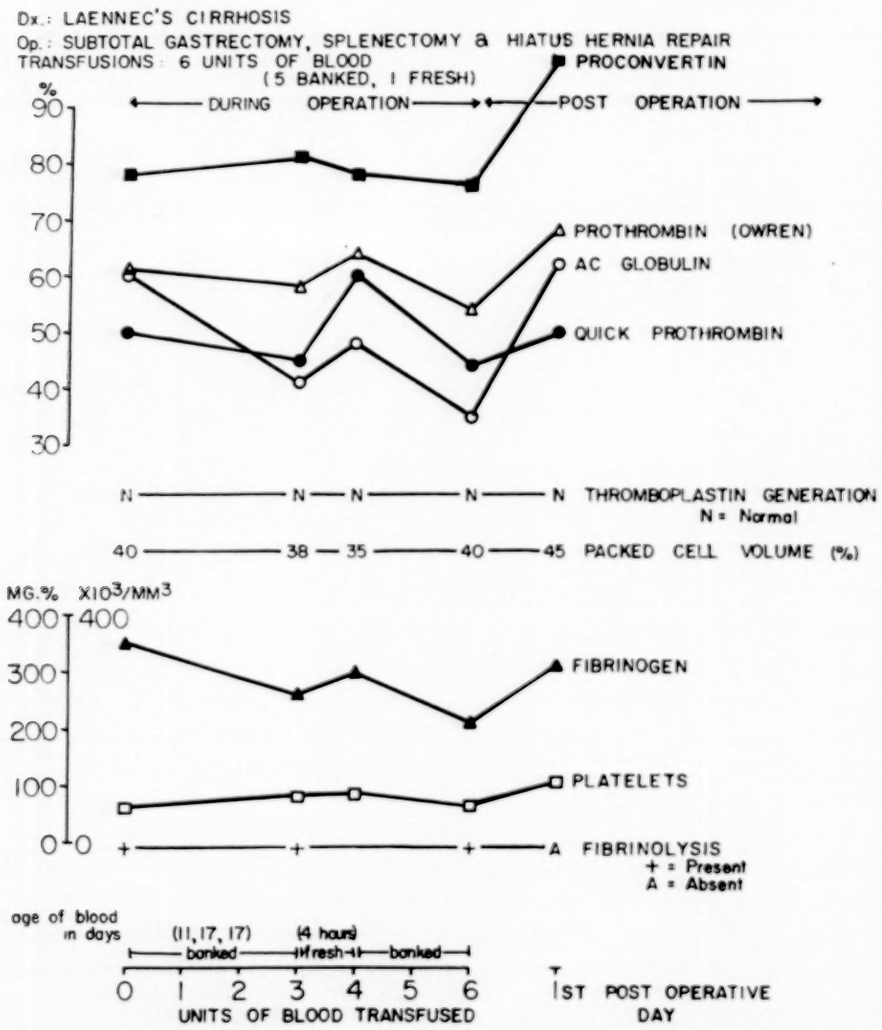

FIG. 6. Effect of transfusions of bank blood and fresh blood on coagulation factors during surgery. Preoperative levels of prothrombin and proconvertin were not altered during transfusion of 5 units of bank blood and 1 of fresh blood. However, Ac-globulin fell during the infusion of bank blood, except for a slight elevation from 1 unit of fresh blood. There was no significant change in platelet level and fibrinolysis was present throughout the operative period.

addition hypofibrinogenemia developed. In the other patient (Case IX) complete fibrinolysis in two hours was present preoperatively and the plasma fibrinogen was 350 mg. per cent. The rate of lysis did not alter throughout surgery, and no change in fibrinogen level occurred. In the other three patients abnormal lysis not present preoperatively appeared during surgery (complete in nine, twelve and twenty-four hours) without development of hypofibrinogenemia. All the patients with increased fibrinolysis during surgery underwent splenectomy, a procedure reported to cause temporary acceleration of the fibrinolytic mechanism in cirrhosis of the liver [*23*]. All but three of the thirteen operations included splenectomy. However, increased fibrinolysis has been found to follow excitement, exercise, anesthesia and any major surgical procedure [*23*].

It should be emphasized that fibrinolysis may occur without any significant fall in plasma fibrinogen. Whether or not a hemorrhagic diathesis may result from fibrinolysis without accompanying hypofibrinogenemia is debatable, although this has been described [*24,25*]. It should also be noted that fibrinolysin destroys coagulation factors other than fibrinogen, namely accelerator globulin, prothrombin, anti-

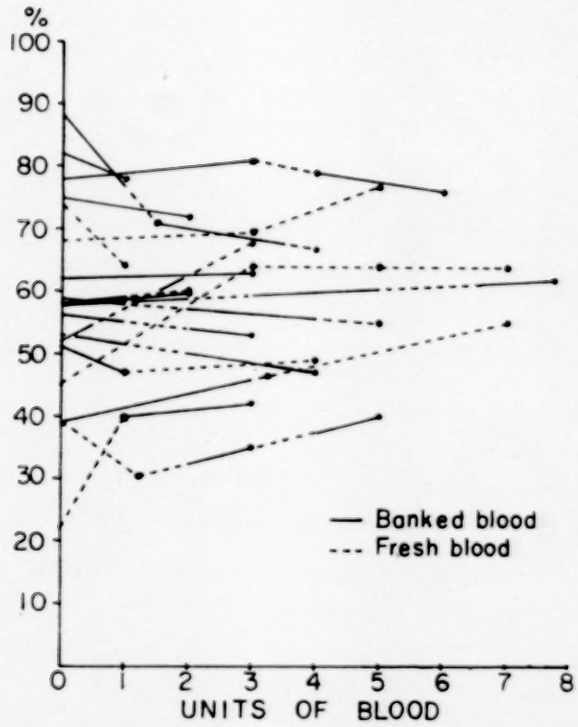

FIG. 7. Effect of serial blood transfusions on proconvertin concentration in patients with liver disease.

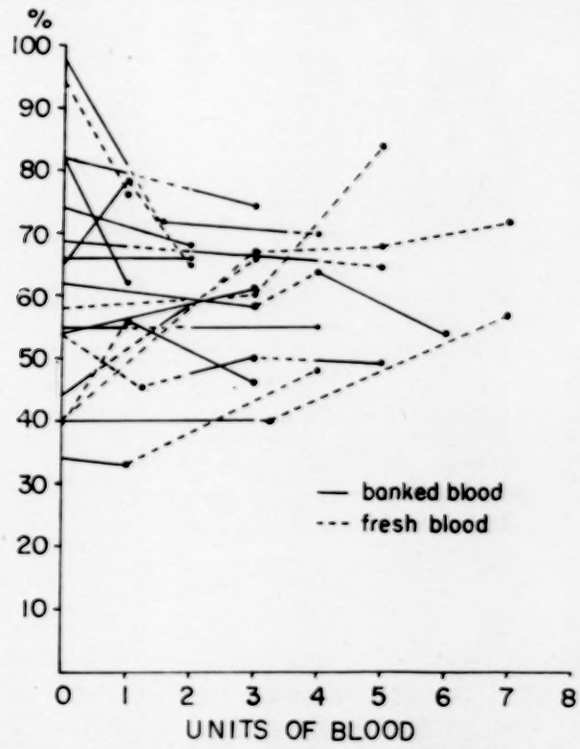

FIG. 8. Effect of serial blood transfusions on prothrombin (Owren) concentrations in patients with liver disease.

hemophilic factor and possibly proconvertin [*26,27*]. The development of fibrinolysin in a patient with liver disease will therefore increase the existing coagulation deficiencies.

Postoperative Studies. In the small number of patients studied postoperatively, clotting defects, partially corrected during surgery and transfusion, recurred rapidly, usually by the first to third postoperative day. (Fig. 3.) This is consistent with the known short half-life of these coagulation factors *in vivo*. In those patients in whom the transfusions caused a depression of coagulation factors the preoperative levels were restored within twenty-four to forty-eight hours. (Fig. 6.) Patient IX (Table II), whose platelet count rose from 57,000 to 347,000/cu. mm. ten days after splenectomy, was observed during the subsequent ten months. Two months after splenectomy her platelet count was 70,000/cu. mm. and remained at this level except during periods of cortisone administration, when the platelet count rose to normal levels. This suggests that in addition to hypersplenism some other mechanism (? autoimmune) may play a role in the thrombocytopenia in liver disease.

Postsplenectomy Platelet Response (eleven patients, Fig. 10). The platelet response following splenectomy was that of a gradual, progressive increase. This lends support to the concept that the thrombocytopenia of liver disease is due at least in part to hypersplenism. Five thrombocytopenic patients became normal; three showed slight thrombocytosis (600,000/cu. mm.). Three patients with low platelet counts initially showed improvement but did not become normal. Two of these patients died in the postoperative period as a consequence of recurrent hemorrhage and hepatic coma. The surviving patient showed a platelet rise from 72,000 to 165,000/cu. mm. In the only patient with a normal platelet count prior to splenectomy (extrahepatic portal hypertension) mild thrombocytosis developed postoperatively (580,000/cu. mm.). Pronounced thrombocytosis following splenectomy was not observed in this group of patients.

Incidence of Abnormal Bleeding during and after Surgery. In many of the cases included in this study, abnormal but controllable oozing was noted at operation even when hypotensive spinal anesthesia was used. Death from uncontrollable hemorrhage during surgery did not occur in any of the cases. However, two patients were re-explored for massive postoperative intra-abdominal hemorrhage. Both these pa-

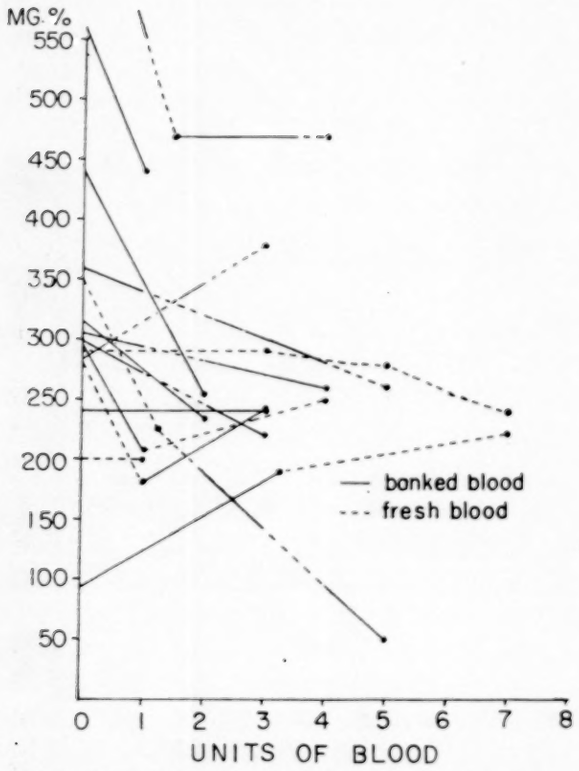

FIG. 9. Effect of serial blood transfusions on fibrinogen concentration in patients with liver disease. The marked fall in fibrinogen occurred in a patient who demonstrated increased fibrinolysis during operation.

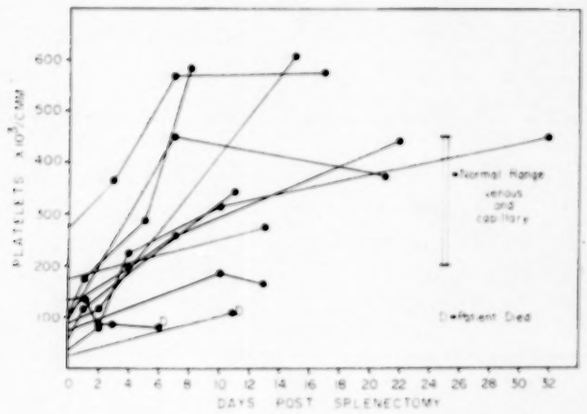

FIG. 10. Platelet response after splenectomy in patients with liver disease.

tients died. In one (Case XVI) the cause of the bleeding was found to be arterial hemorrhage from a malignant hepatoma, and was spontaneous or caused by operative trauma. Although the hemorrhage was stopped at exploration, massive gastrointestinal hemorrhage subsequently developed and the patient died in hepatic coma. Spontaneous hemorrhage from hepatomas has been seen previously at this hospital [*28*]. In the other patient (Case VII) increased fibrinolytic activity and hypofibrinogenemia developed during surgery and there was massive bleeding into the peritoneal cavity in the immediate postoperative period. In spite of correction of the hypofibrinogenemia by transfusion of blood and fibrinogen and subsidence of fibrinolytic activity, it was apparent that bleeding continued and at re-exploration forty-eight hours after initial surgery abnormal oozing of blood was noted in the subcutaneous, omental and splenic bed tissues. The bleeding was controlled by local hemostatic measures and the signs of intra-abdominal hemorrhage abated after the second operation. Eight days later (ten days following initial shunt surgery) massive gastrointestinal hemorrhage developed and the patient died in hepatic and renal failure. The degree of hypofibrinogenemia and fibrinolysis could have been responsible for the early postoperative hemorrhage, but could not have been the cause of the diffuse oozing at the time of the second laparotomy, as the fibrinogen level was then 370 mg. per cent and no fibrinolysis was demonstrable in the whole blood. Suture of the multiple bleeding points stopped the hemorrhage. The bleeding may have been due to the lysis of clots in small vessels which had been injured but not ligated during the initial surgery and which had continued to bleed after fibrinolytic activity disappeared. A diffuse abnormality of the coagulation mechanism was evident, and this could have been an additional reason for the abnormal bleeding during the second procedure. However, many other patients with similar defects did not bleed. Thus it is difficult to define the exact cause of bleeding in these patients even when complete coagulation studies are available. It is surprising that a greater incidence of hemorrhage did not occur in view of the severity of the coagulation defects in some of these patients.

Evaluation of Coagulation Tests for the Detection of Hypocoagulability in Liver Disease. The prothrombin time proved to be the most useful test for the detection of the coagulation disturbance produced by parenchymatous liver disease. When the prothrombin time was within the normal range, no significant plasma coagulation defects were observed; when the prothrombin time was slightly prolonged (equivalent to 50 per cent prothrombic activity), only mild

deficiency of the affected coagulation factors were noted; when the prothrombin time indicated a prothrombic activity of less than 40 to 50 per cent, significant coagulation factor deficiencies were encountered. In general the decrease in the various plasma factors affected by liver disease paralleled the increase in prothrombin time. This was true not only of the factors known to affect the prothrombin time (prothrombin, proconvertin, accelerator globulin) but also of the serum thromboplastic activity, which includes factors which have no effect upon the prothrombin time, such as PTC. In no case did we observe an abnormality in thromboplastin generation in the absence of an abnormal prothrombin time.

Although in general thrombocytopenia occurred most frequently in those patients with significant plasma coagulation defects and prolonged prothrombin times, this was not always the case. Significant thrombocytopenia was observed in cases in which only mild plasma coagulation defects were found (Cases VIII, IX and XVII). It is therefore essential that platelet counts be taken or careful examination of the peripheral smear be made in order to evaluate the platelets quantitatively. (We made no qualitative evaluation of the platelets. This could be done in part by use of the thromboplastin generation test.)

Clotting times in glass were found of little value in the detection of coagulation abnormality. For the most part they were normal even in the presence of significant thrombocytopenia and depression of several plasma factors. This is to be expected since it is known that in patients with marked thrombocytopenia or with congenital AHF or PTC deficiencies, with levels of 3 to 10 per cent of these factors, normal clotting times are found.

Prothrombin consumption was also found to be of little value in detecting the coagulation defect. This test is relatively insensitive and only in the most severe cases of liver disease might one expect this test to be abnormal. However, in such cases the prothrombin content of the plasma is also markedly reduced (Cases I, XI and XII) and as a result the residual serum prothrombin is low. The calculation of prothrombin consumption is invalid under these circumstances. The thromboplastin generation test is frequently abnormal in liver disease and can be used as a screening test. However, it is not more sensitive than the prothrombin time in detecting the abnormality of coagulation associated with liver disease, and is considerably more time consuming.

Therapy of Hemorrhage during Operative Procedures in Patients with Parenchymatous Liver Disease. As already indicated, the coagulation defect in liver disease is one which involves many factors. In most cases the deficiency of each of the affected factors is mild to moderate and if present as a single abnormality would not result in a break in the hemostatic mechanism even during surgery or trauma. However, the presence of multiple defects alters the situation, for although spontaneous hemorrhage may not occur the stress of surgery or trauma will frequently result in a break in hemostasis, and excessive bleeding. Loss of blood and coagulation of blood during surgery cause a further loss of coagulation factors which the damaged liver cannot readily replace, and thus the coagulation defect is increased. When surgery was first introduced for the relief of portal hypertension in cirrhosis, excessive operative bleeding was not infrequently encountered and deaths occurred despite massive transfusions of bank blood. With the introduction of fresh blood transfusions during surgery the amount of bleeding during surgery and operative mortality due to hemorrhage decreased.

The questions arise as to what factors in fresh blood may account for this beneficial effect and what other forms of therapy might be of value. Three coagulation factors are known to deteriorate during storage of blood at 5°C., namely accelerator globulin, antihemophilic factor and platelets. (Fibrinogen may develop a slight decrease in reactivity to thrombin during storage of blood, but the change is so minor that it probably has little or no significance in the problem under discussion.) Accelerator globulin and platelets are also characteristically depressed in advanced liver disease. The other factors depressed in liver disease, namely prothrombin, proconvertin, PTC, and other thromboplastic factors (Stuart factor, PTA) are relatively stable *in vitro* and are present in satisfactory concentration in bank blood even after twenty-one days' storage.

The advantage of fresh blood would therefore appear to be due to its normal content of Ac-globulin and platelets. Studies reveal that for twenty-four to forty-eight hours after collection of blood in A. C. D. and in any type of container there is little or no loss of Ac-globulin activity. Following this there is a steady loss in activity so

that in general there is a 40–50 per cent loss in one week, 60–70 per cent in two weeks, and 80–90 per cent loss in three weeks. It is evident, therefore, that for replacement of this factor, blood stored up to forty-eight hours is as good as freshly drawn blood, and that blood stored for one week should cause no significant deficiency of this factor in the recipient.

Although the platelet content of stored blood does not decrease very rapidly (10–40 per cent loss in one week, 30–60 per cent in two weeks, 40–70 per cent in three weeks) and the remaining platelets exhibit good thromboplastic activity, viability is rapidly lost. Most of the platelets in A. C. D. blood remain viable for about four hours regardless of the container and thereafter viability decreases steadily so that by twenty-four to forty-eight hours few if any of the platelets are viable and are rapidly removed from the circulation after infusion. Moreover, it has been demonstrated that massive transfusion of stored blood for replacement therapy may be associated with marked thrombocytopenia in the recipient [*21*]. If one desires to raise the circulating platelet level for several days in thrombocytopenic patients, blood less than twenty-four hours old [*22*] or freshly prepared platelet concentrates [*29*] should be used. On the other hand, adequate but temporary hemostasis in a thrombocytopenic person can be achieved without producing a rise in the platelet count by the use of lyophilized [*30*] or stored platelets [*31*]. It is also advisable to use fresh blood if more than 1,000 to 2,000 ml. of blood must be given, in a ratio of at least 1 unit of fresh blood to 2 units of bank blood. Although there is some disagreement concerning the stability of antihemophilic factor in bank blood, most observers believe that there is a steady fall in these factors during storage. Our own data indicate a 50 per cent loss of AHF after one week of storage. As already noted there is no decrease of AHF in the blood of patients, even with severe hepatocellular disease. However, massive transfusion of bank blood may result in a significant decrease in this factor.

If hypofibrinogenemia is present, it is most readily corrected by infusion of fraction I (fibrinogen). By this means 4 to 6 gm. of fibrinogen can be given rapidly in a small volume and in quantities comparable to the fibrinogen content of 1 to 2 L. of plasma or 2 to 4 L. of blood. However, if fibrinolysis is also present, fresh whole blood should be given in addition to fibrinogen since fibrinolysin also destroys accelerator globulin, antihemophilic factor, prothrombin and proconvertin.

SUMMARY

Coagulation studies were performed in twenty-one patients with liver disease, one patient with obstructive jaundice and three patients with extrahepatic portal hypertension.

The coagulation defect in liver disease was found to be a multiple one involving platelets, accelerator globulin, prothrombin, proconvertin, PTC and possibly other unidentified serum and plasma thromboplastic factors. The degree of impairment roughly paralleled the severity of the liver disease. Fibrinolysis of variable degree was observed in 20 per cent of the patients, usually in the presence of normal fibrinogen levels, and in an additional 25 per cent fibrinolytic activity developed during surgery.

The prothrombin time was found to be the best screening test available for the evaluation of the plasma coagulation mechanism of these patients. The platelet depression did not necessarily parallel the depression of the other coagulation factors.

Severe plasma coagulation abnormalities were corrected partially for twenty-four to forty-eight hours by the combined use of fresh and stored blood. The superiority of fresh blood over stored bank blood was concluded to be, to a large degree, the result of the greater concentration of accelerator globulin and the viability of the platelets in fresh blood.

Abnormal oozing was noted during surgery, but uncontrollable hemorrhage was not encountered in the patients studied. Postoperative hemorrhage occurred in two patients, one a spontaneous hemorrhage from a hepatoma and the other associated with hypofibrinogenemia and fibrinolysis.

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The Significance of the Direct-Reacting Fraction of Serum Bilirubin in Hemolytic Jaundice*

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EXCESSIVE hemolysis characteristically raises the serum concentration of indirect-reacting bilirubin, an effect presumably due to overloading of the excretory mechanism of the liver resulting in retention of pigment derived from the breakdown of hemoglobin. The *qualitative* van den Bergh reaction under these circumstances is indirect. However, spectrophotometric analyses of the diazotization reaction indicate that a small but significant fraction of the total serum bilirubin in hemolytic icterus reacts directly [*1–3*]. The biochemical nature and physiological significance of this component have received little attention, so that it is not known whether (1) hemolysis injures the liver, permitting the regurgitation of direct-reacting bilirubin glucuronide from the biliary tree, (2) reflux of bilirubin glucuronide into the blood is a normal phenomenon, the magnitude of which varies with the concentration of pigment in the bile, and, hence, increases when the excretion of bilirubin is enhanced by hemolysis, (3) small amounts of glucuronide are formed in the extrahepatic tissues when the concentration of bilirubin in the serum is elevated, (4) other direct-reacting conjugates of bilirubin are formed in the liver or extrahepatic tissues when the normal glucuronide-conjugating system of the liver is overwhelmed by an excess of unbound pigment, or (5) a significant fraction of unconjugated bilirubin is capable of diazotizing directly, i.e., in the absence of alcohol or other catalytic agents.

The failure of the kidney to excrete bilirubin in hemolytic icterus, which has given rise to the term "acholuric jaundice," may be related to the nature of the pigments present in the serum. Although the factors governing the urinary excretion of bilirubin are still ill-defined, it is generally believed that the serum concentration of the direct-reacting fraction is of paramount importance [*3,4*]. However, in hemolytic icterus the latter often exceeds the normal level without producing bilirubinuria. This raises two questions. First, is the direct-reacting pigment in hemolytic icterus identical with that in hepatocellular and obstructive jaundice, conditions in which bilirubin may appear in the urine when the direct-reacting fraction in the serum is only minimally increased [*3*]? Second, is the excretion of bilirubin in the urine dependent on some other alteration in the serum related to hepatocellular injury or biliary obstruction? It is clear from recent studies [*5,6*] that the direct-reacting pigment of the serum in hepatocellular and obstructive jaundice is the glucuronide of bilirubin normally excreted in the bile. In contrast, the glucuronide has not been found in measurable quantities in the serum of patients with hemolytic jaundice [*7,8*]. However, the analytical procedure is relatively insensitive and may fail to detect the small concentrations to be expected in this condition [*7,8*].

The purpose of the present investigation was to re-examine the nature and significance of the direct-reacting serum pigment of hemolytic jaundice in the light of current concepts of bilirubin metabolism. In particular, an attempt was made to define the role of hepatic dysfunction and the importance of direct diazotization of unconjugated bilirubin in raising the serum concentration of direct-reacting pigment. The study included observations on the correlation between the serum bilirubin pattern and the

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state of hepatic function in patients with hemolytic jaundice, the effects of infused bilirubin on the serum pigments of subjects with and without hepatic disease, and the relationship between the serum pattern and the urinary excretion of bilirubin in hemolytic and experimental hyperbilirubinemia.

MATERIAL AND METHODS

Subjects. The records of all patients with hemolytic disease seen at the Grace-New Haven Community Hospital between 1952 and 1957 were reviewed, but only those with complete hematological data and with well documented evidence indicating the presence or absence of hepatic disease were included in the study. (Table 1.) The hemolytic disorders represented in the group of forty-six cases selected were as follows:

Erythroblastosis fetalis	21
Acquired hemolytic anemia	10
Congenital hemolytic anemia	8
Sickle cell anemia	5
Cooley's anemia	1
Hemolytic transfusion reaction	1

All subjects included had total serum bilirubin concentrations of 1.5 mg. per cent or greater.

Analytical Methods. Serum bilirubin: This was determined by a minor modification of the Malloy and Evelyn method [9] employing a Coleman junior spectrophotometer. Direct-reacting bilirubin was estimated at one minute as suggested by Ducci and Watson [10]. The upper limits of normal for the direct and total serum bilirubin levels in this laboratory are 0.25 and 1.30 mg. per cent, respectively.

Urine bilirubin: Qualitative tests for bilirubin were carried out by the diazo mat (Ictotest®) method [11]. The Thoma and Kitzberger oxidative method [12] was used to estimate the concentration of bilirubin. Values of 0.05 mg. per cent are considered abnormal in this laboratory and correspond to a trace reaction by the qualitative diazo mat method [11].

Liver function tests: Each of the patients studied, with the exception of the infants with erythroblastosis, had the following tests: serum bilirubin, bromsulphalein retention at forty-five minutes following a 5 mg./kg. test dose, cephalin-cholesterol flocculation, thymol turbidity, serum alkaline phosphatase, urine bile and urobilinogen, and fractionated serum proteins. The methods employed have been described elsewhere [13].

Preparation of Bilirubin Solutions for Diazotization Studies. An aqueous stock solution was prepared by dissolving 50 mg. of crystalline bilirubin (Hoffman-LaRoche) in 50 ml. of 0.1 M sodium carbonate and adjusting the pH to 7.1–7.4 by bubbling carbon dioxide through the solution. A 10 ml. aliquot of stock solution was then added to 10 ml. of pooled human serum of low bilirubin content, following which serial dilutions were made with the serum to yield concentrations ranging from approximately 1 to 50 mg. per cent. A correction was made for the bilirubin content of the original serum. A second set of bilirubin solutions of similar concentration was made up in 0.01 N sodium hydroxide and then added to serum.

Preparation of Bilirubin Solutions for Intravenous Injection. Crystalline bilirubin was dissolved in warm 0.1 M aqueous sodium carbonate to yield a final concentration of 400 mg. per cent. The pH was then brought to 7.8–8.2 by bubbling carbon dioxide through the solution for five to ten minutes.

Analysis of Crystalline Bilirubin Solutions to Exclude the Presence of Direct-reacting Glucuronide. The chromatographic method of demonstrating the presence of glucuronide was essentially that described by Partridge [14]. Crystalline bilirubin was dissolved in a 0.1 M aqueous sodium carbonate solution at a concentration of 200 mg. per cent and adjusted to a pH of 7.2–7.4 with carbon dioxide. Aliquots were diazotized in the presence of methanol as described by Malloy and Evelyn [9], extracted with re-distilled butanol, concentrated in a flash evaporator at 40°c., and then hydrolyzed by heating at 100°c. for one hour with 2 N hydrochloric acid. Samples containing up to 1,500 μg. of diazo pigment were applied to Whatman 3 MM filter paper and chromatographed by the descending technic for four to six hours in a mobile solvent system composed of butanol, acetic acid, and butyl acetate (20:100:80). Samples of unhydrolyzed diazo pigment and of hydrolyzed phenolphthalein glucuronide (Sigma Chemical Company) were applied to the same paper as controls. After air-drying, the papers were dipped into a 1:1 solution of 0.1 N silver nitrate and 5 N ammonium hydroxide and then dried in an oven at 105°c. for five minutes. The typical brown or black spots denoting the presence of glucuronic acid were obtained with hydrolyzed phenolphthalein glucuronide, but not with the hydrolyzed or unhydrolyzed azo pigment. The method was found to be sensitive to 3.8 μg. of glucuronic acid (equivalent to 10 μg. of hydrolyzed phenolphthalein glucuronide). If all the crystalline bilirubin had been present in the form of the diglucuronide the theoretical yield of glucuronic acid on hydrolysis of 1,500 μg. would have been 645 μg. Hence, it is evident that if the crystalline bilirubin used in these experiments contained any of the glucuronide, its concentration must have been less than 0.6 per cent as the diglucuronide or 1.0 per cent as the monoglucuronide.

RESULTS

Direct-reacting Serum Bilirubin in Hemolytic Disorders. As demonstrated in Figure 1, 77 per cent of the 108 values for direct-reacting bilirubin obtained in forty-six patients with hemolytic disorders exceeded the upper limit of normal of

TABLE I

DIAGNOSES, SERUM AND URINE LEVELS OF BILIRUBIN, AND EVIDENCE OF HEPATIC DISEASE IN FORTY-SIX PATIENTS WITH HEMOLYTIC JAUNDICE

Case No.	Diagnosis	Serum Bilirubin		Direct: Total Ratio (%)	Urine Bilirubin (Ictotest)*	Evidence of Hepatic Disease
		Direct (mg. %)	Total (mg. %)			
1	Cooley's anemia	0.32	1.76	18.2	—	Liver biopsy: extensive bile stasis
		0.20	2.80	7.1	—	
2	Sickle cell anemia	0.20	4.50	4.4	0	None
3	Sickle cell anemia	0.65	4.94	13.2	—	None
		0.30	2.34	12.8	—	
		5.34	10.8	49.4	4+	Liver biopsy: sickle cell hepatitis
4	Sickle cell anemia	0.22	2.02	10.9	2+	None
		0.14	1.80	7.8	—	
		0.19	1.55	12.3	—	
5	Sickle cell anemia	0.08	2.70	3.0	—	None
		0.18	2.32	7.8	—	
6	Sickle cell anemia	0.31	1.80	17.2	0	None
		0.23	1.60	14.4	—	
		0.65	2.18	29.8	—	
7	Congenital hemolytic icterus	0.60	4.00	15.0	—	None
8	Congenital hemolytic icterus	0.29	3.22	9.0	1+	None
9	Congenital hemolytic icterus	0.25	5.66	4.4	—	None
10	Congenital hemolytic icterus	0.21	2.70	7.8	—	None
11	Congenital hemolytic icterus	1.33	7.30	18.2	—	None
12	Congenital hemolytic icterus	0.19	2.54	7.50	—	None
13	Congenital hemolytic icterus	0.19	5.52	3.4	0	None
		0.29	7.56	3.8	0	
14	Congenital hemolytic icterus	0.56	3.20	17.5	0	None
		0.14	1.92	7.3	0	
		0.19	3.20	5.9	—	
15	Acquired hemolytic anemia	0.28	2.64	10.6	0	None
		0.20	1.68	11.9	0	
		0.12	2.16	5.6	—	
		0.32	2.86	11.2	—	
		0.30	1.68	17.9	—	
16	Acquired hemolytic anemia	0.20	3.14	6.4	—	None
		0.10	2.58	3.9	—	
17	Acquired hemolytic anemia	0.64	2.88	22.2	—	None
		0.56	2.22	25.2	—	
18	Acquired hemolytic anemia	0.31	2.58	12.0	—	None
		0.63	5.82	10.8	0	
		0.31	1.56	20.0	1+	
19	Acquired hemolytic anemia	0.40	1.80	22.2	0	None
20	Acquired hemolytic anemia	0.24	2.12	11.3	0	None
21	Acquired hemolytic anemia	0.40	2.32	17.2	—	None
22	Acquired hemolytic anemia	0.31	2.34	13.2	—	None
23	Acquired hemolytic anemia	0.59	10.08	5.9	0	None
		0.55	1.82	30.2	1+	
24	Acquired hemolytic anemia	0.45	10.70	4.2	1+	None
		0.08	2.00	4.0	0	
		0.49	9.14	5.4	0	
		0.37	9.32	4.0	0	
		0.80	16.10	5.0	1+	
		0.90	6.00	15.0	1+	
25	Transfusion reaction	0.23	1.82	12.6	—	None
26	Erythroblastosis fetalis	1.20	42.10	2.9	—	None
		0.60	15.10	4.0	—	
		0.60	26.80	2.2	—	
		0.45	9.60	4.7	—	
		0.39	15.60	2.5	—	
27	Erythroblastosis fetalis	0.80	21.00	3.8	—	None
		0.43	19.00	2.3	—	
		0.55	16.00	3.4	—	
28	Erythroblastosis fetalis	0.75	9.60	7.8	—	None
		0.91	9.70	9.4	—	
		0.40	11.10	3.6	—	
		0.25	9.60	2.6	—	

TABLE 1 (*Continued*)

DIAGNOSES, SERUM AND URINE LEVELS OF BILIRUBIN, AND EVIDENCE OF HEPATIC DISEASE IN FORTY-SIX PATIENTS WITH HEMOLYTIC JAUNDICE

Case No.	Diagnosis	Serum Bilirubin		Direct: Total Ratio (%)	Urine Bilirubin (Ictotest)*	Evidence of Hepatic Disease
		Direct (mg. %)	Total (mg. %)			
29	Erythroblastosis fetalis	1.65	24.30	6.8	—	None
		1.55	21.90	7.1	—	
30	Erythroblastosis fetalis	0.37	9.14	4.0	—	None
31	Erythroblastosis fetalis	0.44	10.50	4.2	—	None
		0.40	11.68	3.4	—	
32	Erythroblastosis fetalis	0.30	12.04	2.5	—	None
		0.22	15.00	1.5	—	
		0.18	18.60	1.0	—	
33	Erythroblastosis fetalis	0.55	4.77	11.5	—	None
34	Erythroblastosis fetalis	0.60	16.20	3.7	—	None
		0.70	16.60	4.2	—	
		0.55	18.40	3.0	—	
35	Erythroblastosis fetalis	0.64	19.70	3.2	—	None
		0.98	20.80	4.7	—	
36	Erythroblastosis fetalis	0.45	12.60	3.6	—	None
37	Erythroblastosis fetalis	0.48	4.44	10.8	—	None
38	Erythroblastosis fetalis	0.82	10.80	7.6	—	None
39	Erythroblastosis fetalis	2.50	15.40	16.0	—	Hepatomegaly: liver to umbilicus
40	Erythroblastosis fetalis	2.17	9.16	23.7	—	Hepatosplenomegaly
41	Erythroblastosis fetalis	1.26	12.40	10.1	—	None
42	Erythroblastosis fetalis	1.82	10.40	17.5	—	Hepatosplenomegaly
		1.24	6.00	20.6	—	
		3.38	13.40	25.2	—	
		3.84	13.40	28.7	—	
		3.40	11.00	30.9	—	
		1.64	4.24	38.7	—	
43	Erythroblastosis fetalis	2.80	28.70	9.8	—	Liver biopsy: giant cell hepatitis
		4.30	26.30	16.3	—	
		7.00	16.00	43.8	—	
		3.45	5.50	62.7	—	
44	Erythroblastosis fetalis	0.36	14.10	2.6	—	None
		0.75	30.50	2.5	—	
		0.40	19.60	2.0	—	
		0.44	18.60	2.4	—	
		0.20	6.30	3.2	—	
		0.40	16.70	2.4	—	
45	Erythroblastosis fetalis	0.40	3.50	11.4	—	None
		0.90	7.40	12.2	—	
		0.90	9.10	9.9	—	
		0.75	8.40	8.9	—	
46	Erythroblastosis fetalis	0.20	18.40	1.1	—	None
		0.60	24.10	2.5	—	
		0.20	17.90	1.1	—	
		0.75	22.50	3.3	—	
		0.45	20.60	2.2	—	
		0.80	23.90	3.3	—	

* — = No test performed.
0 = Negative for bilirubin.
+ = Positive for bilirubin.

0.25 mg. per cent. It is noteworthy that thirteen of the eighteen concentrations above 1.20 mg. per cent and all those above 1.75 mg. per cent occurred in the six patients with definite clinical, laboratory or pathological evidence of concomitant hepatic disease. (Table 1.) In accord with the observations of Zieve and his associates [*2*], the direct-reacting fraction constituted less than 15 per cent of the total serum bilirubin in most of the patients with uncomplicated hemolytic disease, and more than 15 per cent in all but one of those with complicating hepatic disease. However, this distinction was not apparent at levels of total serum bilirubin

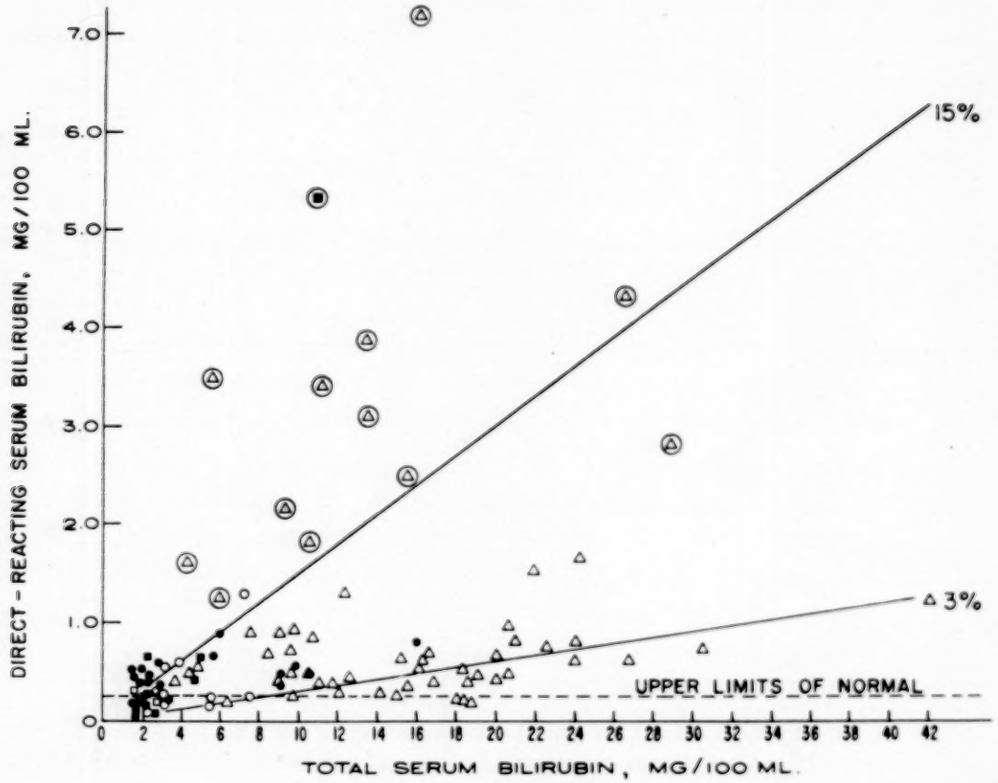

FIG. 1. Relationship of direct-reacting to total serum bilirubin in forty-six patients with hemolytic jaundice. △—erythroblastosis fetalis, ▲—hemolytic transfusion reaction, □—Cooley's anemia, ■—sickle cell anemia, ○—congenital hemolytic anemia, ●—acquired hemolytic anemia. Symbols enclosed within large circles indicate complicating hepatic disease.

below 4.0 mg. per cent, the percentage of direct-reacting pigment exceeding 15 per cent in nine of the thirty-six instances of uncomplicated hemolytic hyperbilirubinemia in this range. Similarly, in normal subjects the fraction often exceeds 15 per cent. This may be related to the inaccuracy of the analytical method at very low concentrations of direct-reacting pigment.

Considering the group as a whole there appeared to be no correlation between the levels of direct-reacting and total bilirubin, but when the values for patients with and without complicating hepatic disease were analyzed separately it was found that there was a statistically significant positive correlation between direct and total bilirubin in the subjects with hepatic disease ($r = 0.91$, $p = <0.01$), but none in the cases of uncomplicated hemolysis ($r = 0.2$, $p = 0.99$).

It is evident from these data that excessive hemolysis is capable of raising the concentration of direct-reacting bilirubin in the serum to a maximum of 1.2 mg. per cent, the level being determined, in part at least, by the concentration of total bilirubin, and that concentrations above this value usually denote the presence of complicating hepatic disease.

Direct Diazotization of Unconjugated Bilirubin. To explore the possibility that the increase in the direct-reacting fraction of serum bilirubin in patients with hemolytic disease was due to the diazotization of unconjugated pigment in the absence of alcohol, the behavior of unconjugated crystalline bilirubin was examined. Preliminary analysis of this pigment failed to demonstrate the presence of any bilirubin glucuronide. However, the method was not sufficiently sensitive to exclude the presence of the monoglucuronide in amounts constituting less than 1.0 per cent of the total pigment (see Methods). The results of duplicate determinations of direct-reacting and total bilirubin in serial dilutions of crystalline bilirubin dissolved in 0.1 M sodium carbonate and added to normal serum at the pH of the blood are plotted in Figure 2. It will be observed that a small but significant fraction (2.66 ± 0.48 per cent) of the pigment diazotized in the absence of

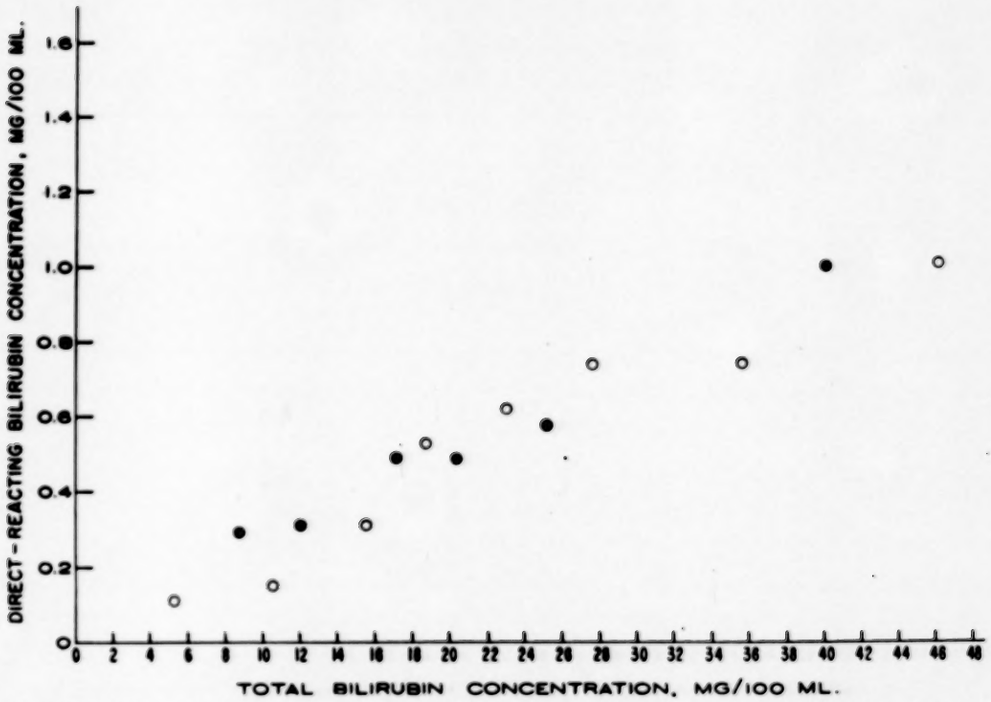

FIG. 2. Fraction of crystalline bilirubin in 0.1 M sodium carbonate (●) and 0.01 N sodium hydroxide (○) that reacted directly within one minute, showing its relationship to the concentration of pigment in solution.

alcohol or other catalytic agents, and that the absolute amount was proportional to the concentration of bilirubin in solution. Virtually identical results (2.31 ± 0.47 per cent) were obtained with crystalline bilirubin dissolved in 0.01 N sodium hydroxide added to serum. (Fig. 2.) On the basis of these results, and allowing for the maximum amount of contaminating glucuronide that could have escaped detection, it may be estimated that at least 0.7 and as much as 3.0 per cent of unconjugated bilirubin is capable of reacting directly within one minute. As is evident in Figure 1, there were seventeen serums in which the direct-reacting fraction constituted less than 3 per cent of the total bilirubin, an amount consistent with the hypothesis that all the pigment in hemolytic icterus is of the unconjugated variety. However, in ninety-one instances the fraction was greater than 3 per cent, so that in these serums some glucuronide or other direct-reacting conjugate of bilirubin must have been present. Fifteen of the samples were obtained from the six patients with evidence of complicating hepatic disease, which may have permitted the regurgitation of bilirubin glucuronide from the bile. A similar mechanism could have been involved in the remaining forty cases if the liver contained lesions that escaped detection. However, two other possibilities must be considered: (1) that, even in the absence of hepatic damage, bilirubin glucuronide may gain access to the blood from either the parenchymal cells or the bile ducts when the amount produced in the liver is greatly enhanced as a consequence of excessive hemolysis, and (2) that bilirubin may be conjugated in the extrahepatic tissues when its concentration in the serum is high. Accordingly, an attempt was made to investigate these mechanisms by examining the behavior of intravenously injected crystalline bilirubin in subjects with and without hepatic disease.

Behavior in the Serum of Intravenously Injected Crystalline Bilirubin. Five control subjects and six patients with histologically-confirmed cirrhosis were given an intravenous infusion of 0.4 per cent crystalline bilirubin in 0.1 M sodium carbonate brought to a pH of 7.8–8.2 with carbon dioxide. A total of 15 mg./kg. of body weight was administered over a twenty to sixty minute period. The controls included two normal subjects and three patients with asymptomatic gastrointestinal disease in whom physical examination and laboratory investigation failed to

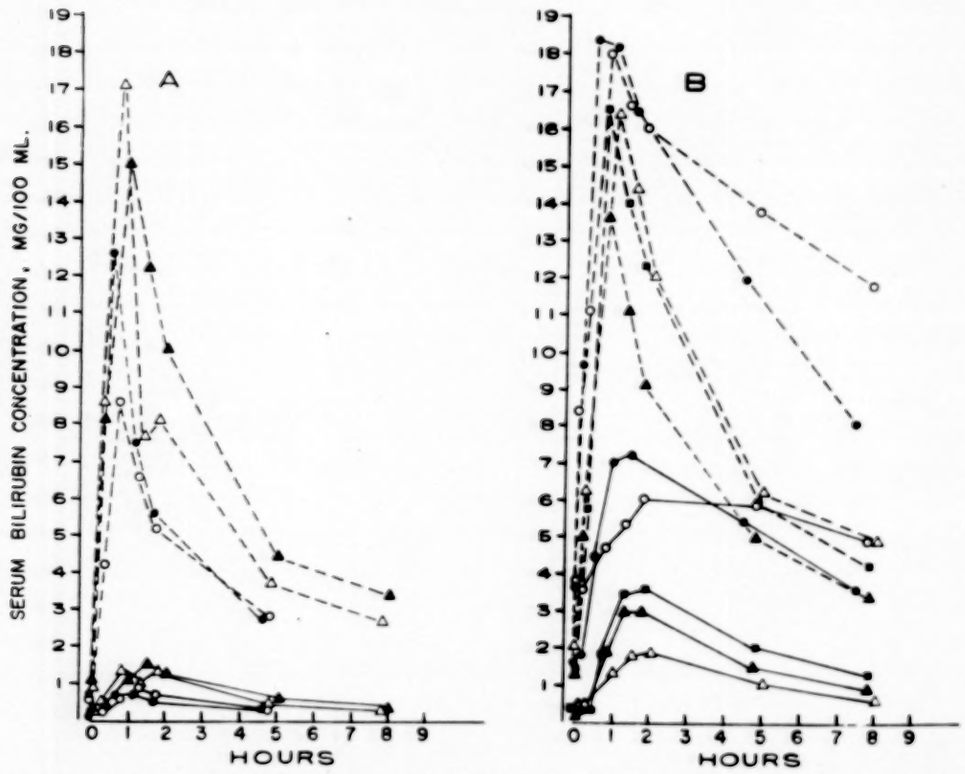

FIG. 3. Levels of direct-reacting (—) and total (--) serum bilirubin in normal (A) and cirrhotic (B) subjects infused with crystalline bilirubin (15 mg./kg. body weight). The infusions were started at zero hours and were continued for a period of one-half to one hour. Symbols indicated refer to subjects listed in Table II. A, normal subjects: ●—R. O., ○—G. R., ▲—G. I., △—C. O. B., cirrhotic subjects: ●—M. I., ○—C. L., ▲—B. L., △—D. E., ■—P. A.

TABLE II

DIAGNOSES, CONTROL AND POSTINFUSION SERUM BILIRUBIN LEVELS IN SUBJECTS RECEIVING INTRAVENOUS INFUSIONS OF CRYSTALLINE BILIRUBIN (15 MG./KG. BODY WEIGHT)

Patient	Diagnosis	Control Serum Bilirubin		Maximum Serum Bilirubin After Infusion	
		Direct (mg. %)	Total (mg. %)	Direct (mg. %)	Total (mg. %)
	Control Subjects				
R. O.	Peptic ulcer	0.05	0.68	0.70	12.36
G. R.	Gastric polyp	0.00	1.10	0.84	8.57
G. I.	No disease	0.00	0.53	1.50	15.00
C. O.	Chronic pancreatitis	0.00	0.86	1.22	17.14
F. I.	No disease	0.09	0.65	2.29	18.34
	Subjects with Hepatic Disease				
M. I.	Laennec's cirrhosis	1.61	3.67	7.24	18.72
C. L.	Laennec's cirrhosis	3.70	8.40	6.04	18.00
B. L.	Biliary cirrhosis	0.17	1.32	3.00	13.60
D. E.	Laennec's cirrhosis	0.26	1.99	1.88	16.37
P. A.	Laennec's cirrhosis	0.28	1.44	3.60	16.51
G. A.	Laennec's cirrhosis	0.20	0.91	5.49	19.13

reveal evidence of hepatic disease. Of the six patients with hepatic disease, five had active Laennec's cirrhosis and one had inactive secondary biliary cirrhosis. With one exception, all the patients with cirrhosis had some degree of hyperbilirubinemia initially, but in only two was it of sufficient magnitude to produce clinical jaundice. (Table II.)

The changes in the concentration of direct-reacting and total serum bilirubin during and following the infusion of unconjugated crystalline bilirubin are plotted in Figure 3. In all subjects there was a sharp rise in the concentration of total bilirubin, with a peak coinciding invariably with the end of the infusion. Thereafter, the concentration fell at a somewhat slower rate, so that the levels were still elevated at the end of eight hours. The maximum increase in concentration was not significantly different in the two groups (13.4 ± 4.5 mg. per cent in the control subjects, and 12.7 ± 5.5 per cent in the patients with cirrhosis). However, as might be expected, the clearance rate, judging from

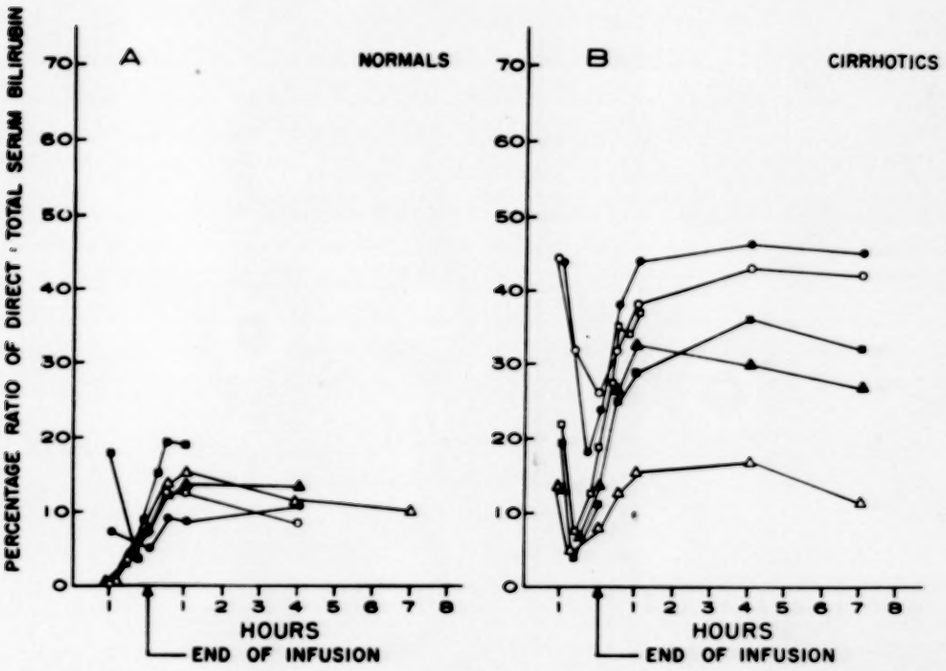

FIG. 4. Serial changes in the ratio of direct-reacting to total serum bilirubin in the normal (A) and cirrhotic (B) subjects receiving infusions of crystalline bilirubin illustrated in Figure 3.

the percentage decrease in bilirubin concentration in the four hours following the end of the infusion, was significantly greater in the control group than in the group with cirrhosis (73.4 ± 5.4 and 52.7 ± 13.7 per cent, respectively; $t = 2.79$, $p = <0.05$).

As is evident in Figure 3, the rise in total bilirubin was invariably accompanied by an increase in the direct-reacting fraction. However, there were two striking differences: (1) the increment was significantly greater in the cirrhotic subjects than in the control subjects (3.5 ± 1.5 and 1.3 ± 0.5 mg. per cent, respectively; $t = 3.13$, $p = <0.02$), and (2) in both groups the peak concentration did not coincide with that of the total bilirubin, but occurred one-half to one hour later. At its maximum, the increment in direct-reacting bilirubin constituted from 8.0 to 19.6 (average 12.9) per cent of the coincident increment in total bilirubin in the control subjects, and from 16.1 to 44.0 (average 32.2) per cent in the patients with cirrhosis. Since, as shown previously, not more than 3.0 per cent of the injected bilirubin reacted directly when added to serum *in vitro*, it must be assumed that in both groups a fraction of the pigment was converted to one of its direct-reacting conjugates, presumably the glucuronide, following its infusion. That the increase in direct-reacting pigments, particularly in the group with cirrhosis, was not due to the presence in the serum of an activating agent that facilitated the diazotization of unconjugated bilirubin in the absence of alcohol is evident from the observation that the peaks for direct-reacting and total bilirubin did not coincide in time, and from the results of previously reported experiments [*15*] indicating that the *in vitro* addition of unconjugated bilirubin to direct-reacting jaundiced serum did not alter its behavior in the van den Bergh reaction.

The fact that cirrhotic subjects converted a much larger fraction of the injected bilirubin to a direct-reacting pigment than the control subjects makes it highly improbable that conjugation was accomplished in the extrahepatic tissues and suggests that the conversion occurred in the liver, following which some of the glycuronide gained access to the circulation, an effect that hepatic injury might be expected to exaggerate. Indeed, the behavior of the direct-reacting : total bilirubin ratio during and after the infusion supports this concept, and suggests that the degree of regurgitation was determined by the functional and anatomical status of the liver. Thus, it will be observed in Figure 4 that, with the exception of an initial fall due to the rapid infusion of pigment whose reaction was

predominantly indirect, the percentage of direct-reacting bilirubin promptly returned to its initial value, or slightly above, both in the cirrhotic patients and in the control subjects, and was independent of the concentration of total bilirubin which was approximately the same in both groups. (Fig. 3.) As in the case of hemolytic disorders (Fig. 1), the ratio tended to remain below 15 per cent in those with normal livers and above 15 per cent in those with hepatic disease.

Since the infusion of unconjugated bilirubin into subjects without hepatic disease was followed by the appearance in the serum of direct-reacting pigment, presumably as a consequence of regurgitation, it is reasonable to postulate that the same mechanism was responsible for the increase in the conjugated fraction of serum bilirubin observed in patients with hemolytic disorders. The apparent absence of conjugated bilirubin in many of the serums obtained from infants with erythroblastosis fetalis, as evidenced by the finding of direct:total bilirubin ratios of less than 3 per cent in seventeen of the fifty-eight samples tested (Fig. 1), appears to be inconsistent with the hypothesis that regurgitation occurs when bilirubin production is increased. However, considering the fact that the capacity of the liver to conjugate [*16*] and excrete [*17*] bilirubin is very limited in the neonatal period, the concentration of bilirubin glucuronide in the bile of these infants may not have been sufficiently high to permit significant regurgitation. If, as implied in this interpretation, the amount of bilirubin glucuronide regurgitated in hemolytic disorders uncomplicated by hepatic disease is dependent on its concentration in the bile rather than on the level of total bilirubin in the serum, it might be anticipated that the relationship between direct-reacting and total bilirubin in the serum would be a variable one, dependent, in part at least, on the efficiency of pigment excretion. This may account for the scatter of ratios observed in Figure 1.

The possibility that the appearance in the serum of direct-reacting pigment following the infusion of crystalline bilirubin was due to the reflux of bilirubin glucuronide from the parenchymal cells of the liver rather than from the bile cannot be excluded with certainty. However, if the conjugation of bilirubin is the limiting factor in its excretion, as suggested by many investigators [*18*], the latter would appear to be more likely. Similarly, the lag of one-half to one hour between the peak concentration of total bilirubin attained at the end of the infusion and that of direct-reacting pigment is more consistent with the regurgitation of bilirubin from the bile, considering the delay that its conjugation and excretion and subsequent equilibration with the blood might entail.

In an attempt to demonstrate more directly the uptake and subsequent regurgitation of bilirubin in the liver following its infusion, simultaneous measurements of direct-reacting and total serum bilirubin in arterial and hepatic venous blood were made in one normal and one cirrhotic subject. Catheterization of the hepatic vein was carried out under fluoroscopic control in the usual manner [*19*]. The infusion solution was identical with that used in the previously described experiments. As in the latter, the peak concentrations of total bilirubin coincided with the end of the infusion and preceded that of the direct-reacting fraction by approximately one-half hour. (Fig. 5.) It will be observed that in both subjects the level of total bilirubin was slightly higher in arterial than hepatic venous blood throughout most of the experiment, suggesting a significant uptake of bilirubin by the liver. Whether the few exceptions were due to technical errors or to reversal of the direction of bilirubin movement is uncertain, but the former appears more likely. Neither the AV difference nor the peak concentration of total bilirubin differed significantly in the two subjects, from which it may be inferred that the capacity of the liver to abstract bilirubin from the circulation was not impaired as a consequence of the cirrhotic process. In contrast, the concentration of direct-reacting pigment rose to a higher level in the cirrhotic subject, as in the previously described experiments (Fig. 3), suggesting that regurgitation was enhanced in the presence of hepatic injury. The normal subject showed no significant AV difference in direct-reacting bilirubin concentration, which raises the question whether or not regurgitation could have occurred via the lymph, as in early obstructive jaundice [*20*]. Unfortunately, these data do not permit any conclusion on this point. Assuming that the normal subject had a hepatic blood flow of 1.5 L. per minute, a blood volume of 5 L. and a hematocrit of 40 per cent, an increase in bilirubin concentration of as little as 0.12 mg. per cent in hepatic venous blood could theoretically have accounted for the 2.2 mg. per cent increase in arterial concentration that

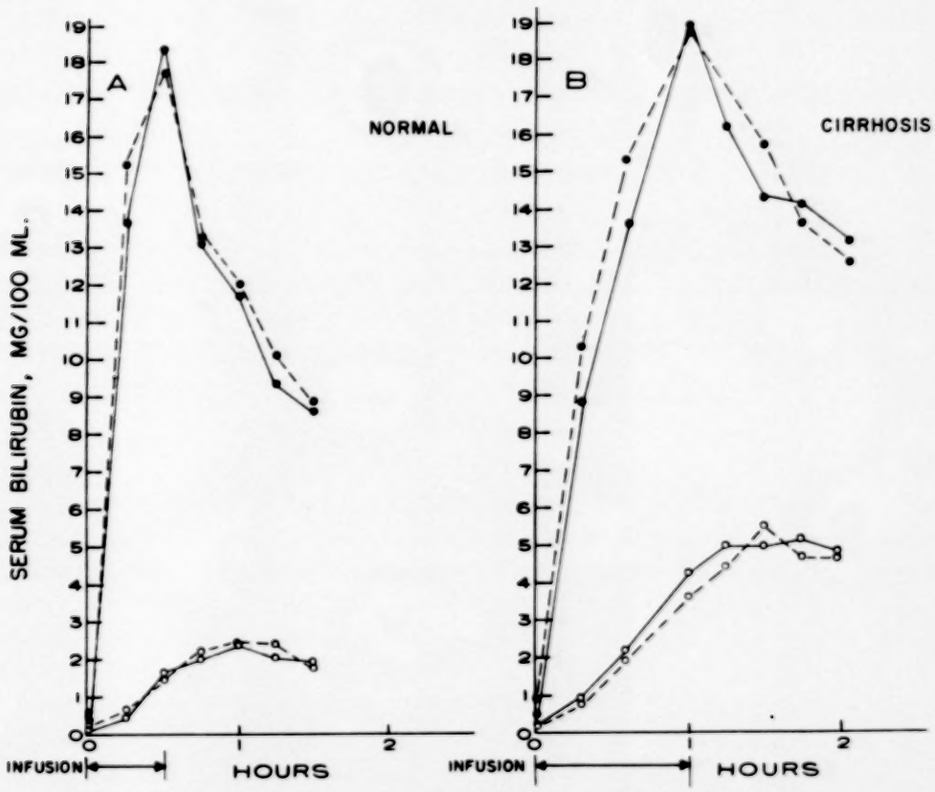

FIG. 5. Direct-reacting(○) and total (●) serum bilirubin levels in the hepatic vein (—) and in a peripheral artery (--) of normal subject F. I. and cirrhotic subject G. A. during and following infusion of crystalline bilirubin (15 mg./kg. body weight). Note that the peak concentration of total bilirubin coincided with the termination of the infusion, but that the peak concentration of direct-reacting occurred about one hour later.

occurred during the first hour. However, an indeterminate amount of pigment was lost in the tissues, as evidenced by the appearance of slight jaundice, so that no estimates can be made of the concentration in hepatic venous blood that would be expected if all the direct-reacting pigment was regurgitated into the sinusoids or periportal capillaries of the liver. In the cirrhotic subject the concentration of direct-reacting bilirubin in hepatic venous blood was consistently, with one exception, 0.2 to 0.6 mg. per cent higher than that in arterial blood. This difference is consistent with the concept that conjugated bilirubin was regurgitated into the hepatic venous blood, but does not exclude the possibility that some of the pigment entered the circulation via the lymph. On the basis of the same assumptions and calculations made in the normal subject, this difference could theoretically have accounted for the 5.3 mg. per cent increase in the arterial concentration of direct-reacting bilirubin that occurred during the first hour and a half. However, this would make no allowance for the loss of pigment in the tissues and urine that undoubtedly took place, therefore it is again possible that additional direct-reacting pigment reached the blood by way of the lymph.

Bilirubinuria in Hemolytic Disorders and Following Infusions of Crystalline Bilirubin. Qualitative tests by the diazo mat (Ictotest) method [*11*] revealed the presence of bilirubin in eight of the twenty-three urine specimens collected from patients with hemolytic disorders in whom simultaneous serum bilirubin determinations were carried out. Of the positive tests, one occurred in the sample obtained from a subject with evidence of complicating hepatic disease, and seven in the twenty-two samples obtained from subjects without hepatic disease. As is evident from the data in Table I, there was no threshold of either direct-reacting or total serum bilirubin at which bilirubinuria invariably appeared.

In order to examine more closely the relation-

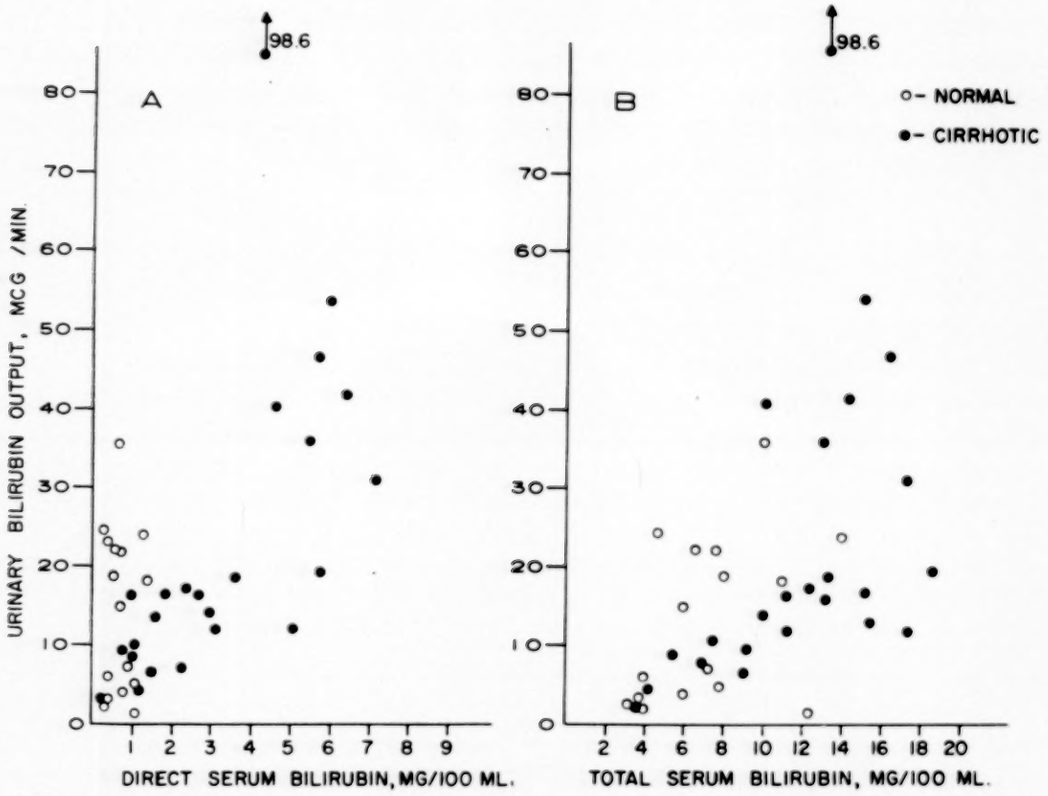

FIG. 6. Relationship between the amount of bilirubin excreted in the urine and the concentrations of direct-reacting (A) and total (B) serum bilirubin in normal (○) and cirrhotic (●) subjects during and following an infusion of crystalline bilirubin (15 mg./kg. body weight).

ship between bilirubinuria and the level of the serum pigments, and to determine whether or not the presence of hepatic disease affected the renal excretion of bilirubin, quantitative measurements of urinary bilirubin excretion were made in the normal and cirrhotic subjects previously described in whom crystalline bilirubin was infused. Voided urine specimens were collected within a minute or two of blood sampling at each of the intervals indicated in Figure 3, and their content of bilirubin compared with the mean concentrations of direct-reacting and total serum bilirubin calculated from the values found at the beginning and end of each collection period. All subjects were found to excrete bilirubin at a concentration in excess of 0.05 mg. per cent, the level at which the qualitive diazo mat test becomes positive [*11*], during and for a period of five to seven hours following the infusion. Although there was considerable overlapping of values, the concentration tended to be higher in the cirrhotic subjects (0.1 to 3.16 mg. per cent) than in the control subjects (0.08 to 0.50 mg. per cent). However, it is evident from Figure 6, which shows the relationship between the amount of bilirubin excreted and the concentrations of direct-reacting and total bilirubin in the serum, that the excretion in the cirrhotic subjects was greater only in those instances in which the serum concentrations were higher than in the control subjects, suggesting that the presence of hepatic disease had little effect on the renal excretion of bilirubin. Indeed, the renal clearance of bilirubin (UV/P) proved to be lower in the cirrhotic subjects (0.2 to 1.3 cc./minute) than in the control subjects (0.1 to 8.0 cc./minute). Of particular interest is the fact that while the rate of bilirubin excretion tended to increase as its concentration in the serum rose, in neither group was the rate any more closely correlated with the serum level of the direct-reacting fraction than with that of total bilirubin. This was true not only of the group as a whole (Fig. 6), but also of the serial observations in individual subjects.

The failure to observe a close relationship between the amount of bilirubin excreted and

the concentration of direct-reacting serum bilirubin in these experiments appears to be inconsistent with the results of recent studies [*5*] indicating that most if not all the urinary bilirubin is in the form of a glucuronide. However, it is realized that the technics used for measuring bilirubin changes in the present study were relatively crude, in that complete urine collections were not obtained by catheterization and studies were carried out during periods when serum concentrations of bilirubin were changing rapidly. Hence, no definite conclusions regarding the mechanisms governing the renal excretion of bilirubin are warranted from these observations. Nevertheless, they indicate clearly that bilirubinuria often occurs when a sufficiently large amount of unconjugated bilirubin is introduced into the circulation, presumably due to the occurrence in the serum of some direct-reacting bilirubin.

COMMENT

The results of the present investigation are somewhat at variance with the traditional view that hemolytic icterus is a form of pure retention jaundice due to the accumulation of unconjugated bilirubin derived from the breakdown of hemoglobin and produced in excess of the excretory capacity of the liver. Although the qualitative indirect van den Bergh reaction of the serum supports this concept, the evidence presented indicates that the serum often contains a small but significant fraction of conjugated bilirubin presumably regurgitated from the bile. Thus, it was shown that while 3 per cent or less of the unconjugated bilirubin in serum is capable of diazotizing directly (i.e., in the absence of alcohol) within one minute, the amount found in hemolytic disease not infrequently exceeds this value. In some instances, this can be related, in part at least, to the presence of complicating hepatic disease, but in others this does not appear to be a factor, which suggests that, even in the absence of liver damage, conjugated bilirubin may be regurgitated from the bile when the excretion of pigment is greatly accelerated. Consistent with this interpretation is the observation made in this study that the infusion of crystalline bilirubin into subjects with normal livers is followed by the appearance in the serum of direct-reacting bilirubin in amounts exceeding 3 per cent of the injected pigment. That this is not due to the presence in the serum of a catalytic agent that facilitates the diazotization of unconjugated bilirubin in the absence of alcohol is evident from the fact that the concentration of direct-reacting serum pigment does not reach a peak for at least one-half to one hour following the conclusion of such infusions. Although it was possible to demonstrate the regurgitation of conjugated bilirubin directly in a cirrhotic patient infused with crystalline bilirubin by showing that the concentration of direct-reacting pigment was higher in the hepatic vein than in a peripheral artery, it was not possible to do so in a normal subject. However, it was pointed out that the theoretical AV difference required to account for the increase in direct-reacting pigment in the periphery was so small it fell within the error of the analytical procedure employed. Moreover, evidence was cited to show that a significant fraction of the pigment regurgitated in the liver may reach the blood via the lymph.

The amount of conjugated bilirubin that regurgitates in hemolytic disease is variable. However, in the serum it seldom exceeds 15 per cent of the total bilirubin, and when it does it usually signifies the presence of complicating hepatic disease. It is noteworthy in this connection that the fraction of direct-reacting bilirubin found in the serum following an infusion of bilirubin was also less than 15 per cent in normal subjects and over 15 per cent in patients with hepatic disease.

In some forms of hemolytic disease 3 per cent or less of the serum bilirubin reacts directly, indicating the absence of conjugated pigment. This is seen most frequently in erythroblastosis fetalis, a condition occurring during neonatal life when the capacity of the liver to conjugate [*16*] and excrete [*17*] bilirubin is limited. Similarly low direct:total serum bilirubin ratios have been reported in non-hemolytic constitutional hyperbilirubinemia, another disorder in which bilirubin conjugation and excretion are subnormal [*21,22*]. This suggests that the fraction of bilirubin in the serum that reacts directly is more closely correlated with the amount of pigment conjugated and excreted than with the concentration of total pigment in the serum, and is consistent with the suggestion that at least some of the direct-reacting fraction in the serum in hemolytic disease is regurgitated from the bile.

Recent studies [*5,6*] indicate that bilirubin is excreted as a glucuronide and that the direct-reacting fraction of serum bilirubin in obstruc-

tive and hepatocellular jaundice is similarly conjugated. It is reasonable to suppose, therefore, that the pigment presumed to be regurgitated in hemolytic disease is also a glucuronide. Admittedly most attempts to demonstrate the presence of bilirubin glucuronide in the serum of such patients have been unsuccessful [*7,8*]. However, the technics employed for this purpose are relatively insensitive and are incapable of quantitating the amounts expected in this disease [*7,8*]. An alternative but less likely possibility that cannot be excluded with certainty is that the direct-reacting serum pigment in hemolytic disease is some other water-soluble conjugate of bilirubin formed in the liver when its normal glucuronide-conjugating system is overwhelmed by an excess of pigment. That such conjugates may be produced is suggested by the recent demonstration that a fraction of the direct-reacting bilirubin in the bile of experimental animals is present in the form of a sulfate conjugate [*23*].

The absence of bilirubinuria is generally considered one of the highly characteristic features of hemolytic icterus. However, it is clear from the evidence presented here that bilirubinuria does occur occasionally even in patients without complicating hepatic disease. Since bilirubin is excreted in the urine in the form of glucuronide [*5*], it is reasonable to suppose that the occurrence of bilirubinuria in patients with hemolytic disease and in normal subjects infused with crystalline bilirubin is related, in part, to the appearance in the serum of direct-acting pigment. Although no correlation between the amount of bilirubin in the urine and the concentration of direct-reacting pigment in the serum was evident in the present study, it is recognized that the technics employed were inadequate to either establish or exclude such a relationship.

SUMMARY

An attempt has been made to elucidate the nature and significance of the direct-reacting fraction of serum bilirubin in hemolytic jaundice by examining (1) the relationship between the pattern of serum bilirubin, the presence of bilirubinuria and the state of hepatic function in forty-six patients with hemolytic disorders, (2) the quantitative van den Bergh reaction of crystalline bilirubin dissolved in serum, and (3) the effect of infusing crystalline bilirubin on the serum pattern and urinary excretion of bilirubin in subjects with and without hepatic disease.

The observations reported indicate that (1) in hemolytic jaundice the direct-reacting fraction usually constitutes less than 15 per cent of the total serum bilirubin, and rarely exceeds 1.2 mg. per cent unless there is accompanying hepatic dysfunction, (2) approximately 3 per cent of the unconjugated bilirubin in the serum is capable of diazotizing in the absence of alcohol within one minute, and hence accounts for a variable fraction of the direct-reacting pigment depending on the concentration of unconjugated bilirubin present, (3) the direct-reacting fraction present in excess of 3 per cent of the total serum bilirubin probably represents bilirubin glucuronide regurgitated from the bile, (4) such regurgitation occurs in the normal liver when the excretion of bilirubin glucuronide is greatly accelerated by excessive hemolysis or the infusion of large amounts of unconjugated bilirubin, and is enhanced when hepatic disease is a complication, (5) the fraction of direct-reacting pigment found in the serum under these circumstances is more closely correlated with the amount of bilirubin excreted in the bile than with its concentration in the serum, and hence tends to be very low in conditions such as uncomplicated erythroblastosis fetalis, in which conjugation and excretion are subnormal, and (6) bilirubinuria is seen occasionally in uncomplicated hemolytic jaundice, but is a regular finding in normal subjects infused with large amounts of crystalline bilirubin.

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The Natural History of Esophageal Varices*

A Study of 115 Cirrhotic Patients in Whom Varices Were Diagnosed Prior to Bleeding

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In the recent literature some authors have suggested that the indications for portal shunt surgery should be broadened to include patients who have varices secondary to cirrhosis of the liver even though no bleeding has yet occurred. Welch et al. [*1*] and Palmer et al. [*2*] have been quite definite in their affirmation of this indication for surgical intervention. That this view is not accepted without reservation is also apparent. Child [*3*] and Nachlas et al. [*4*] have questioned the wisdom of advocating such an approach. A recent editorial [*5*] expressed the opinion that the position of decompression surgery as a prophylactic measure should remain *sub judice*.

Those who support this surgical indication apparently base their opinion on (1) the mortality of 50 to 80 per cent which has been reported for the first year after hemorrhage occurs; and (2) an assumption that the majority of patients with varices are destined to bleed seriously. It is apparent that before the ultimate accomplishments of prophylactic shunt surgery can be evaluated, the incidence of bleeding and death from hemorrhage occurring in the natural clinical course of comparable patients must be known. Since little factual information is available relative to the clinical course of such patients, it seemed worthwhile to make a study of a series of patients in whom the clinical course was uninterrupted by surgery. It was hoped that answers to the following questions might be forthcoming: (1) What is the incidence and characteristics of bleeding among these patients? (2) What is the mortality from bleeding, particularly that due to the first hemorrhage? (3) What is the course of the patients in whom bleeding does not occur?

In such a study it is important that the occurrence of bleeding be adequately documented. Furthermore, it is necessary to determine, as nearly as possible, the origin of bleeding; this cannot always be accomplished but it is essential to classify the hemorrhages according to the weight of evidence supporting a variceal etiology. It is apparent that the relationship of hemorrhage to death should be clearly indicated.

The inference that the mortality of 50 to 80 per cent occurring during the first year after variceal bleeding may be, for the most part, amenable to prophylactic portal decompression is misleading, since deaths from all causes are included in these reports in the literature. In assigning the cause of death a distinction must be made between those patients in whom bleeding is a final episode in the course of progressive hepatic failure and those in whom hemorrhage occurs while hepatic function is not greatly impaired. In the former the course has already been determined by hepatic deterioration and even though bleeding from varices could be prevented by surgery no significant changes in the ultimate outcome would result. Since shunt operations are directed toward the prevention of hemorrhage, it follows that only those deaths directly due to bleeding, or hepatic failure precipitated by bleeding, can be considered preventable by surgery. Only those patients in whom varices are diagnosed in the presence of an adequate hepatic reserve can be considered to be reasonable candidates for prophylactic shunt surgery.

The point of greatest importance is the mortality from the first episode of bleeding. To establish the validity of prophylactic shunt surgery it must be demonstrated that the mortal-

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ity from the first hemorrhage is of such magnitude as to justify the risks of prophylactic shunt surgery.

It is also desirable to know more about the duration of life and causes of death of those patients with varices in whom bleeding has not occurred. To our knowledge, no such study has been published although the need for such information has been mentioned in the literature. Previous studies have dealt with the course of patients with varices who have bled but have said little about those who have not bled.

MATERIAL AND METHOD

Since 1949 detailed data on 1,140 cases of liver disease have been collected, arranged on punch cards, and filed. Over 600 of the patients reported on had esophagoscopy examinations. It is from this group that the cases in this series were compiled. All cards in this file which were coded as indicating varices demonstrated on esophagoscopy without a history of hemorrhage were pulled and listed, the chart was examined, and the data obtained authenticated by one or more of us. We consider that this group is representative of the cases seen routinely at this large general hospital during the six years from July 1, 1949 to July 1, 1955. The date of assembly of these data was July 1, 1956; therefore, no patient was included whose data had not been in our files for over one year subsequent to the diagnosis of varices. Patients who had an x-ray diagnosis of varices but were not examined by esophagoscopy were not included in this series because in our experience the endoscopic method is least subject to question [*6,7*]. Criteria for grading the severity of the esophageal varices were as follows:

0 = no definite varices visible
1+ = one or more varices under 4 mm. in diameter and under 4 cm. in extent
2+ = multiple varices, 4 to 10 cm. in extent
3+ = multiple varices over 10 cm. in extent

The diagnosis of cirrhosis of the liver was based on history, physical examination, liver function tests and in some cases liver biopsy. In some the diagnosis was confirmed at autopsy. Cases in which there was any question of diagnosis were discarded.

Criteria for the occurrence of bleeding were as follows: "Hematemesis" or "melena" was recorded when it occurred while the patient was on the ward, or prior to admission, and was substantiated by witnesses or accompanied by a red blood cell count less than 3 million per cu. mm. or by shock with a normal erythrocyte count followed by a sharp fall in count, or by a count of 3 million which rapidly rose without blood replacement. "Bleeding per history only" was recorded when a history of hematemesis or melena (which included a description of pitch black stools accompanied by the symptoms of hypovolemia) was obtained. Other bleeding episodes recorded by history only were ignored since, in our experience, these generally turn out not to be true bleeding.

TABLE 1
RATE OF ACCRUAL OF PATIENTS PER YEAR (JULY 1, 1949 THROUGH JULY 1, 1955)

	Entered into Series as of Date Varices were Diagnosed						
Year	7-1-49 to 7-1-50	7-1-50 to 7-1-51	7-1-51 to 7-1-52	7-1-52 to 7-1-53	7-1-53 to 7-1-54	7-1-54 to 7-1-55	Total
No. of patients	10	28	14	20	21	22	115

Criteria for the consideration of bleeding from esophageal varices were as follows: "Unequivocal" when the upper gastrointestinal x-ray series or postmortem examination showed a normal stomach or duodenum, and varices and hematemesis were present; "presumptive" when bleeding was from the upper gastrointestinal tract and there was no history suggestive of peptic ulcer; and "probable" when there was severe upper gastrointestinal hemorrhage in a patient with advanced liver disease, but x-ray films were not obtained, autopsy was not performed, and a good history was not obtainable. The bleeding site was considered "unknown" when varices and ulcer both were present.

An attempt was made to follow-up each patient by personal contact, by letter if contact was not possible, or otherwise simply by positive information that the patient was alive or had died. The status of all patients was determined as of the date of assembly of these data, and if dead the cause of death was ascertained in all cases. There were 115 cases which fulfilled the criteria of selection.

In considering the cause of death a concerted effort was made to determine as far as possible from the charts or from our own personal experience with the patients whether death was due to hemorrhage alone, hepatic insufficiency alone, or a combination of hemorrhage and hepatic insufficiency. In those cases in which death was due to a combination of hemorrhage and insufficiency a further attempt was made to subdivide into those in which hemorrhage was the cause, those in which insufficiency was the primary event, and those in which hemorrhage and insufficiency appeared to be equal factors in the cause of death. In the last category the deaths were charged to bleeding.

This group of patients was accumulated over a period of six years, from July 1, 1949 to July 1, 1955. The patient was entered into the series as of the date varices were first diagnosed. Table 1 indicates the number accrued per annum (July 1 to July 1). The date of assembling the follow-up data on these patients was July 1,

TABLE II
INCIDENCE OF BLEEDING

Total patients	115	100%
No. who bled	33	28.6%
2 hemorrhages	9	7.8%
3 hemorrhages	2	1.7%

TABLE III
SOURCE OF HEMORRHAGE

Patients who bled	33	100%
Source unequivocal	26	78.7%
Source presumptive	5	15.1%
Source unknown	2	6%

1956, therefore the twenty-two most recently acquired patients (July 1, 1954 to July 1, 1955) had been followed up at least one year. Of the total of 115 patients ninety-three were followed up for two years, seventy-two for three years, fifty-two for four years, thirty-eight for five years, and ten for six years. The average follow-up interval for the 115 cases was 3.3 years.

RESULTS

Of the total 115 patients, bleeding occurred in thirty-three. This incidence of 28.6 per cent is much lower than some accounts in the literature would lead one to expect. In seven of these cases hemorrhage was a terminal event in patients already critically ill from hepatic failure. There were twenty-two patients who had one episode of bleeding, of which 5 bled as a terminal event in hepatic failure. Of the seventeen patients who survived the first hemorrhage, eleven subsequently bled. Two of these bled three times. In two instances the bleeding was a final event in hepatic failure.

In twenty-six patients the bleeding was considered to be "unequivocally" due to esophageal varices. In five patients the bleeding was classified as presumptive, in two patients it was classified as of unknown origin. Of the latter, one patient had extensive varices but succumbed one week after hemorrhage from a perforated gastric ulcer and peritonitis. The second patient had minimal varices and duodenal ulcer. He bled three times, the terminal hemorrhage being associated with severe hepatic failure.

The elapsed time from the diagnosis of varices to the first hemorrhage varied from 1 to 187 weeks. It was of interest to find that of the thirty-three instances of first hemorrhage twenty-three occurred within one year and thirty within two years of the initial observation of varices. The fact that only three patients bled for the first time more than two years after varices were diagnosed may have an important clinical implication, namely, that in a patient who has survived one year or more with no bleeding after the diagnosis of varices the chances of hemorrhage are small. If substantiated by further observations, this is an important point in considering a shunt operation in this group of patients.

TABLE IV
CAUSES OF DEATH

Cause of Death	No.	Patients (%)	Deaths (%)
Hemorrhage	20	17.3	27
1st hemorrhage	11	9.5	14.8
Hepatic failure	31	26.9	41.8
Other causes	23	20	31

Seventy-four of the original 115 patients died within the period of study. Twenty deaths were due to exsanguination, comprising 27 per cent of the total number of deaths or 17.3 per cent of the total series. Hemorrhage occurred in seven additional patients as a terminal event in patients succumbing to hepatic failure. These patients would have lived no more than a few days or weeks had no hemorrhage occurred, and could not have been salvaged by surgery performed at an earlier date. Of particular importance is the fact that eleven patients or 9.5 per cent of the total series succumbed to the first hemorrhage. These accounted for 14.8 per cent of all deaths. As previously stated, this is the figure of greatest importance in evaluating the indication for prophylactic surgery to prevent bleeding.

The ominous significance of bleeding when it occurs is borne out by this series. While only 33.3 per cent of the patients died as a direct result of the first incident, only two of the thirty-three who bled were still alive. Among the twenty-two patients who bled only once, eleven died of bleeding and five of hepatic failure, while six survived the bleeding. Of the nine patients who bled twice, eight died of exsanguination and one primarily of hepatic failure. Two patients bled on three occasions, one died of hemorrhage, the other of hepatic coma. Of the total of thirty-three patients who bled, twenty died of bleeding

and seven died of hepatic failure, constituting 79.3 per cent of the group.

Among the seventeen who survived the first hemorrhage, eleven or 61.1 per cent bled again, and all these died. Two died in coma and nine from exsanguination. There were four who died of unrelated causes, three of cardiac disease, 20, 30 and 137 weeks after the first hemorrhage. The fourth succumbed to a perforated peptic ulcer fifty-three weeks later. Two patients were still living. Thus, while it is apparent that the outlook for those patients who bled was poor, it is also apparent that nearly one-third died of causes unrelated to hemorrhage.

Thirty-one patients died of hepatic failure. Seven of these had a terminal hemorrhage which did not alter the already established fatal prognosis. One patient died in coma 115 weeks, and another 197 weeks subsequent to diagnosis of varices. It is evident that hepatic failure was a more common cause of death than hemorrhage. This incidence of hepatic failure is also an indication that serious hepatic disease was not uncommon among these patients, even in the absence of bleeding.

There were twenty-three patients who died from causes other than hemorrhage or hepatic failure. Two of these deaths were directly related to the existing cirrhosis. One patient died of hemorrhage complicating needle biopsy, another died of superimposed hepatoma. Thirteen died of heart disease, two of cerebrovascular accidents, two of unrelated carcinoma, one had a perforated gastric ulcer, one pulmonary embolus, one ruptured abdominal aneurysm, and one pneumonia.

The clinical course of these patients with respect to the cause of death as related to the elapsed time since varices were diagnosed is indicated in Table v. Of the seventy-four deaths, thirty-eight occurred within the first year after varices were diagnosed. Thirty of these deaths were due to hemorrhage or to hepatic failure. Approximately 80 per cent of the total deaths occurred within two years after the diagnosis, while 90 per cent of the deaths due to cirrhosis occurred in this period. This preponderance of deaths during the first two years of follow-up is due to the fact that hemorrhage and hepatic failure were the most frequent causes of death during this period. Of the total deaths due to hemorrhage, 85 per cent occurred during this period while 93.4 per cent of the total deaths due to hepatic failure occurred during the same period. Only 56.5 per cent of the deaths from other causes occurred during this corresponding period. This indicates that the longer these patients survive, the less important liver disease becomes as a cause of death.

TABLE V

SEVENTY-FOUR DEATHS DIVIDED ACCORDING TO CAUSE IN RELATION TO ELAPSED TIME FROM DIAGNOSIS OF VARICES

Cause of Death	1st Yr.	2nd Yr.	3rd Yr.	4th Yr.	5th Yr.	6th Yr.	Total
All causes	38	21	8	5	1	1	74
	51.3%	28.3%	10.8%	6.7%	1.3%	1.3%	100%
Hemorrhage	11(7)	6(2)	2(2)	1	0	0	20
	55%	30%	10%	5%	0	0	100%
Hepatic failure	19	10	1	1	0	0	31
	61.2%	32.2%	03.2%	03.2%	0	0	100%
Other causes	8	5	5	3	1	1	23
	34.7%	21.7%	21.7%	13%	4.3%	4.3%	100%

Figures in parentheses represent death from first hemorrhage.

The survival curve (solid line, Fig. 1) again emphasizes the critical nature of the first two years following the diagnosis of varices. The contour of the curve is strikingly similar to that constructed by Patek et al. [*8*] showing the great mortality during the first two years. The curves are similar in that they show deaths from all causes among a group of cirrhotic patients. It is apparent that the deaths due primarily to exsanguination were less frequent in the Patek series, which probably is a reflection of the fact that the demonstration of varices was not required for inclusion.

The broken line curve (Fig. 1) represents the percentage survival of the thirty-four patients who bled and had been followed the indicated number of years. Only 26.5 per cent were alive after one year. Although the number of cases is small, the curve nearly duplicates those of Ratnoff and Patek [*9*] and Nachlas et al. [*4*] for one year survival.

Comparison of these two curves indicates the fallacy of extending the very poor prognosis of patients with esophageal varices which have bled to patients with esophageal varices diagnosed before bleeding. Only one-third of the latter group died after one year as compared to three-fourths of the former group. To assume that patients with latent varices are inevitably doomed to bleed and necessarily have a very poor prognosis is an erroneous basis for recommending routine prophylactic shunt surgery. As noted in this series the majority of patients escaped bleeding entirely, and less than two-thirds of those who bled died from this cause.

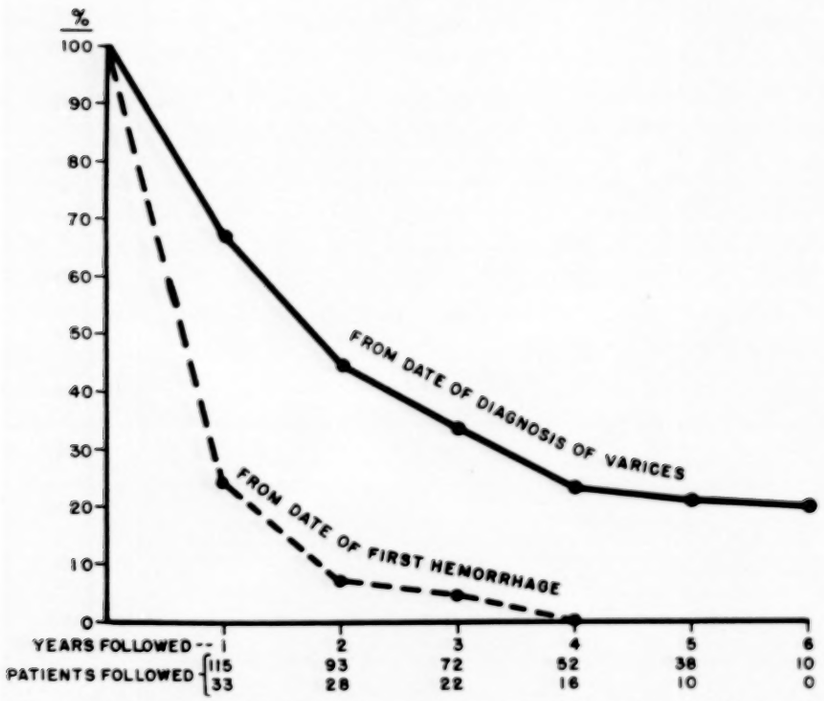

FIG. 1. Per cent survival among patients followed the indicated number of years. (Not to be confused with per cent survival of the total 115 patients.)

The relation between the severity of the varices noted at the time of diagnosis and the incidence of subsequent bleeding is of interest. (Table VI.)

It is evident the largest single group (fifty-one cases) had small varices; however, those of moderate and large size, taken together, outnumbered these. It might be anticipated that small varices would have little clinical significance, but in this series the incidence of bleeding was approximately the same in patients with small varices as in those with varices of moderate size. Hemorrhage did occur nearly twice as frequently among those with large varices as in the other two groups. These findings indicate that varices, even though small, have serious clinical implications.

TABLE VI
SEVERITY OF VARICES CORRELATED WITH BLEEDING*

Severity of Varices	No. of Patients	Patients who Bled					
		No.	Cause of Death				No. of Survivors
			First Hemorrhage	Multiple Hemorrhages	Hepatic Failure	Other Causes	
I	51	13	8	2	1	0	2
II	41	9	2	3	2	2	0
III	23	11	1	4	4	2	0
Total	115	33	11	9	7	4	2

* Varices classified progressively from I to III in order of severity.

The higher incidence of hemorrhage among patients with extensive varices might be assumed to provide a basis for choosing patients for prophylactic decompression. That this is not necessarily true becomes obvious when the mortality is reviewed. Only one of the eleven patients in group III who bled died of the first hemorrhage. This contrasts sharply with group I (small varices) in which eight of thirteen patients died of the first hemorrhage. In fact, the mortality from first hemorrhage was inversely proportional to the extent of the varices throughout this series

When the extent of varices was compared to the deaths due to cirrhosis (hepatic failure and/or hemorrhage) a correlation was evident. This difference is less than might have been expected however; 41.1 per cent of the group with small varices, 46.3 per cent of those with varices of moderate size, and 56.5 per cent of the advanced group having died from these causes. These differences were due to the relative frequency of hepatic failure and may indicate that the severity

of varices is basically correlated with the over-all severity of the existing hepatic disease. These findings also point to the fact that even though varices are small, they are usually associated with relatively severe hepatic disease.

Information as to subsequent changes in the extent of the varices in this series is limited since only twenty-eight patients had repeated esophagoscopic examinations. The previously noted fact that varices may disappear is again verified in that this was noted in nine patients. Varices decreased in extent in seven patients observed, and remained unchanged in six patients. In six instances the varices were more extensive on later examination. This confirms the fact that varices are not in a static state but are constantly subjected to factors which cause changes [*10*].

COMMENTS

The results of this study indicate that there may be need for some change in the prevailing concepts regarding the outlook of cirrhotic patients who have esophageal varices which have not yet bled. It is evident that the idea that nearly all such patients are destined to bleed seriously is not sustained by our data. Only 28.6 per cent of this group had bled up to the time these data were assembled. Thirty-nine patients were still living who had not bled. Of these, twenty-eight had been followed up for over two years since varices were demonstrated. There were eleven who were in the second year of follow-up since diagnosis. All these patients admittedly are still candidates for bleeding. However, in view of the fact that in 91 per cent of those who have had hemorrhages this occurred within two years of the diagnosis of varices, the predictable incidence is very low. An estimate of 10 per cent would seem to be reasonable.

The relatively low frequency of bleeding resulted in a mortality from exsanguination which was lower than might have been expected. This was particularly true with respect to the first episode of bleeding, of which only 9.4 per cent of the total number of patients had died. To this must eventually be added the deaths from first hemorrhage which will occur among the thirty-nine living patients who have not bled. If a rate of mortality comparable to that found among the seventy-four dead patients who had been followed up for similar periods of time is applied to this group, it is fair to estimate there will be one or two deaths from first hemorrhage among these thirty-nine patients. This mortality of slightly over 10 per cent for the 115 patients is most important from the standpoint of evaluating the policy of prophylactic shunt surgery.

It is evident that the prediction that the majority of such patients are destined for serious hemorrhage is not sustained, certainly not the prediction that a large percentage of the total number of patients will succumb to the initial hemorrhage. It is also evident that the mortality statistics gained from the study of patients who have bled from esophageal varices cannot be considered applicable to patients with varices who have not yet bled. The high mortality within one year of bleeding has been cited in support of a similar ominous prognosis for asymptomatic varices. The incidence of hemorrhage among such patients in this series was not sufficiently high to warrant such a conclusion. Furthermore a considerable percentage of the mortality among those who did bleed was not due to exsanguination. Of the thirty-three patients who bled, 78.7 per cent were dead by the end of one year after bleeding began; however, only 68 per cent of these deaths were due to bleeding. Similar percentages are indicated in other series. Nachlas et al. [*4*] found that 46 per cent of the deaths were so caused, Shull [*11*] indicated 45 per cent, while Patek [*8*] reported that only six of twenty-two deaths were due to exsanguination.

It is obvious that the incidence of hemorrhage, and death from hemorrhage, in the natural course of patients with cirrhosis and varices diagnosed prior to bleeding is of prime importance in considering the justification for routine prophylactic shunt surgery in all such patients. The hazard of death from the first hemorrhage together with the fatalities incurred in operating on those who survive this hemorrhage must outweigh the disadvantages of routine operation on all such patients if the policy of prophylactic surgery is to be sustained. These latter factors are (1) mortality from surgery; (2) failure to prevent bleeding; and (3) neuronutritional disturbances following surgery.

The mortality eventuating from surgery in these patients will be dependent to a great extent on the state of hepatic reserve, just as it is in those who have bled. It might be anticipated that patients who have not bled would quite uniformly fall into the "good risk" classification; however, the findings of this analysis would

negate such an assumption. Using the following criteria for "good risk" cases: serum albumin 3 gm. per cent or greater, bromsulphalein retention less than 25 per cent, prothrombin time fifteen seconds or less, cephalin flocculation reaction less than 4 plus, serum bilirubin only slightly elevated and no ascites, there were only forty-one or 35.3 per cent of the patients who qualified at the time varices were first diagnosed. There were twelve other patients (10.4 per cent) who deviated only slightly from the "good risk" group. Fifty-eight or 50 per cent of the patients were considered to be a "poor risk" by these criteria. The condition of some of these patients was such that surgical intervention could not have been seriously entertained. There were twenty-one patients who died within forty weeks after the diagnosis of varices because of severe hepatic failure.

The mortality which has been reported for various series of patients who had bled prior to surgery is variable. Blakemore's [*12*] large experience is quite representative of what has been accomplished. He reports a mortality of 9.4 per cent for "good risk" and 39.6 per cent for "poor risk" patients. Child [*3*] refers to a mortality of 14.2 per cent for fifty-six "fairly good risk" patients. More recently Ebeling et al. [*13*] found a mortality of 15.3 per cent for 104 operations; however, since 1949 the mortality had been reduced to 9.2 per cent. Hallenbeck and Shocket [*14*] had seven deaths among forty-seven patients operated on. There were six immediate postoperative deaths among fifty-seven patients reported by Palmer and Hughes [*15*]. These series include some patients who had not bled prior to surgery, but the incidence of mortality is not segregated. Our experience with patients of all types has resulted in a mortality of over 20 per cent [*16*]. In the paper of Palmer et al. [*2*] it is indicated that eleven patients were operated on prophylactically, with a 11.1 per cent mortality. Child and Donovan [*3*] have suggested that a mortality of between 5 and 10 per cent might be achieved; however, it would seem that this could be reached at the present time only by confining surgery to the "best patients" by the "best risk" surgeons. It is doubtful that a mortality rate as low as 10 per cent could have been reached if all the patients included in present series had been operated on at the time varices were diagnosed.

We can only speculate as to how successful routine prophylactic shunt surgery would be in preventing bleeding. In the literature, recurrent hemorrhage postoperatively among patients who had bled is sufficiently uncommon to support the use of the operation. Recurrent bleeding does occur, as attested to by the report of Ellis et al. [*17*] which reveals an incidence of 14.4 per cent. Blakemore reports 5 of 163 patients undergoing surgery as having died from recurrent bleeding. Linton [*18*] reports a recurrence of bleeding in 17 per cent of seventy-eight patients operated on, while Hallenbeck and Shocket [*14*] had approximately a 20 per cent recurrence rate. In our experience there was a recurrence of bleeding in 31.5 per cent of all patients operated on [*16*]. Rousselot [*19*] recently stated that recurrent hemorrhage was prevented in about 70 per cent of those operated on. Palmer and Hughes [*15*] indicate that eight of fifty-seven patients operated on have bled. It must be kept in mind that these follow-up periods are relatively short in terms of years. While there are no adequate data on patients who have not bled prior to surgery, it would be unrealistic to expect all bleeding to be prevented. In view of the experience cited, it is likely that failures would reach 10 to 20 per cent over a period of years.

That these operations may induce neuronutritional disturbances is established [*20–21*]. While Palmer et al. [*15*] do not consider this important, others [*3b,14,17*] believe this complication may be present in 20 per cent or more of the patients with portocaval shunt. Jones [*22*] believes that surgery places a considerable stress on the cirrhotic patients. Certainly deaths from hepatic failure not infrequently follow closely after shunt surgery. To just what extent the shunt itself may be responsible for this mortality is difficult to determine. It is evident that those advising prophylactic shunt surgery must assume responsibility for the metabolic complications resulting therefrom.

The question immediately arises as to whether or not it is possible to define the patients who are most likely to bleed. A relatively high incidence of subsequent hemorrhage would justify greater surgical risk. Those with the more extensive varices might logically be considered to have the greater bleeding potential. Of the twenty-three patients with severe varices seven were still living, the same number (seven) died from hepatic failure, and five from hemorrhage. In four patients death was due to unrelated causes. Severe hepatic insufficiency was present in three

of the five who died of hemorrhage prior to bleeding. It would therefore seem that a clear-cut possibility of successful prophylactic shunt surgery being life saving was present in only two patients. Of those who were living, two to seven years had elapsed since the diagnosis of varices was made, without occurrence of any bleeding. While this group is small, the indications for prophylactic surgery are no greater than in those with varices of less severity.

The failure to find satisfactory correlations with the severity of the varices is not surprising. Several years ago we drew attention to the variable character of varices [*23*], a finding which has been confirmed by Palmer [*10*]. More recently Palmer [*24*] has indicated that the extent of varices does not correlate well with either their duration or the clinical course of the hepatic disease.

Kagoris [*25*] described the usual anastomosis at the cardia as small, thereby offering considerable resistance to blood flow. He reasoned that very high intravascular pressure for a considerable time was necessary to form sizable varices. It is possible that small varices may have much higher intravascular pressures and bleed more severely than large varices. The findings in this study lend support to this thesis. Palmer [*26*] has also found that the pressure within the varices is not necessarily correlated with their size, and believes that size may be related to the variable distribution of flow through the available collateral beds.

Considerable attention has been given to other factors in the pathogenesis of hemorrhage from esophageal varices. Acid regurgitation, atrophy of the mucosa, perivascular tissue support, peptic ulceration and esophagitis have all been given consideration in this connection [*27–28*]. Until more is known of the reasons for varices bleeding, it is unlikely that reliable criteria for evaluating the propensity to hemorrhage in a given patient with varices can be formulated.

It is our impression that there is a correlation between the severity of liver impairment and the extent of varices, the frequency and severity of bleeding, and the mortality from hemorrhage. The clinical impression that hemorrhage is an accident in the course of cirrhosis does not seem to be true in the majority of instances, but rather it is related to the presence of advanced liver damage. Smythe et al. [*29*] have recently expressed this same concept by observing that in some cases bleeding from varices is a manifestation rather than a cause of increased hepatic failure. Also Allen et al. [*30*] have recently noted that decreased survival time of erythrocytes in cirrhotic subjects is predominantly correlated with esophageal varices. It appears reasonable to assume that the predominant determining factor in relation to all facets of the problem of hemorrhage in cirrhosis is liver damage. It is logical to expect to find all the factors concerned with bleeding to be more evident in those patients with advanced liver disease.

There is one further point which arises in relation to this matter. This series as well as others are compiled from hospitalized patients. While some are discovered to have varices incidentally, the majority are in the hospital because of illness resulting from cirrhosis. The very nature of the clinical course of cirrhosis therefore makes it obvious that in the majority of cases the disease is well advanced and frequently in an acute exacerbation. It might be conjectured that such series do not truly reflect the clinical characteristics of varices as they exist among cirrhotic subjects in the general population. If such information were available, it is possible that the results would indicate that varices have a much less serious connotation than is now attributed to them. This conjecture is based on the premise that the average severity of hepatic damage would be much less.

SUMMARY AND CONCLUSIONS

An analytical study has been made of the clinical course, uninterrupted by surgery, of 115 cirrhotic patients in whom the diagnosis of esophageal varices was established prior to any bleeding. These patients have been followed up for intervals varying from one to six years, average 3.3 years.

Of the 115 patients, forty-one were living and seventy-four were dead at the time the data were assembled. Thirty-three (28.6 per cent) had bled. There were twenty (17.3 per cent) who died of exsanguination, thirty-one (26 per cent) who died of hepatic failure, and twenty-three (20 per cent) who succumbed to unrelated causes. Eleven (9.5 per cent) died of exsanguination during the first episode of bleeding. Approximately 90 per cent of the deaths from liver disease occurred within two years after varices were diagnosed.

Knowledge of these aspects of the natural course of such patients is necessary before it is

possible to know what must be accomplished by routine prophylactic shunt surgery in order to establish the validity of this surgical policy. Inasmuch as the essential difference in the objective of this policy over the practice of reserving surgery for those patients who have bled is directed toward preventing the mortality from the first hemorrhage, this becomes the most important datum in this study. It is estimated that by the time all 115 patients are dead between 10 and 15 per cent will have died of first hemorrhage. This, then, is the ultimate possible goal of achievement, against which must be balanced the mortality, failures and complications of routine prophylactic decompression surgery. It does not seem that at present routine prophylactic decompression surgery offers any definite advantage to the patient. If further experience reveals (1) criteria for determining those patients most likely to bleed; (2) that the over-all surgical mortality can be lowered to approximately 5 per cent; (3) that surgery prevents bleeding in nearly all patients over a period of years; (4) or if it is shown that there are serious remote untoward effects of hemorrhage on the course of the disease, this conclusion may well be unwarranted.

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A Six-Month Evaluation of an Anabolic Drug, Norethandrolone, in Underweight Persons*

I. Weight Gain

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ALTHOUGH anabolic agents are potentially useful in clinical medicine, the compounds heretofore available have been either ineffective or have undesirable side effects which preclude their prolonged use. The anabolic effects of testosterone, and its ability to increase appetite, produce weight gain and induce a feeling of well-being, are well known. However, its androgenic action limits its clinical usefulness, particularly in females and children.

A recently introduced compound, norethandrolone (Nilevar) (17α ethyl-17 hydroxy-norandrostenone) has been shown to be anabolic both for animals [*1,2*] and man [*3–5*]. Animal experiments with this compound have shown an anabolic effect equal to testosterone [*1*] but an androgenic effect only one-sixteenth that of testosterone [*6*]. Short term studies in man have shown its ability to induce a positive nitrogen balance in a variety of clinical situations without significantly affecting sodium balance [*5,7,8*]. Consequently it seemed desirable to evaluate this agent for longer periods of time in essentially healthy persons who were anxious to gain weight.

SUBJECTS

Fifty-four volunteer subjects were studied, all of whom had expressed a desire to gain weight. Many of these subjects had previously used various nutritional supplements without success in gaining weight. All but two were underweight according to standard tables of the Metropolitan Life Insurance Company (1952).

The fifty-four subjects were drawn from three groups. Group I consisted of twenty-eight healthy young workers employed in a large industrial plant. There were ten women and eighteen men ranging in age from eighteen to fifty-six years, with a mean age of twenty-nine.

Group II was composed of sixteen male members of the domiciliary division of a large veterans center. They represented an older population ranging in age from forty-five to seventy years, with a mean age of sixty-three. Many of these men suffered from various chronic disorders such as advanced degenerative joint disease, generalized arteriosclerosis or pulmonary emphysema.

Group III consisted of ten male patients hospitalized on the tuberculosis service of the same veterans center. They ranged in age from nineteen to sixty-seven years, with a mean age of forty-five. All of these patients had demonstrated stability of their disease, as evidenced by stationary weight, negative sputum cultures, absence of fever and normal erythrocyte sedimentation rates over a period of six or more months. All had received antituberculosis drugs for at least six months.

METHODS

A daily oral tablet containing either a placebo or 25 mg. or 50 mg. of norethandrolone was admin-

* From the Research Service, Wood Veterans Administration Center and the Department of Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin. Aided in part by a grant from G. D. Searle and Company, who also supplied the drug as Nilevar.®

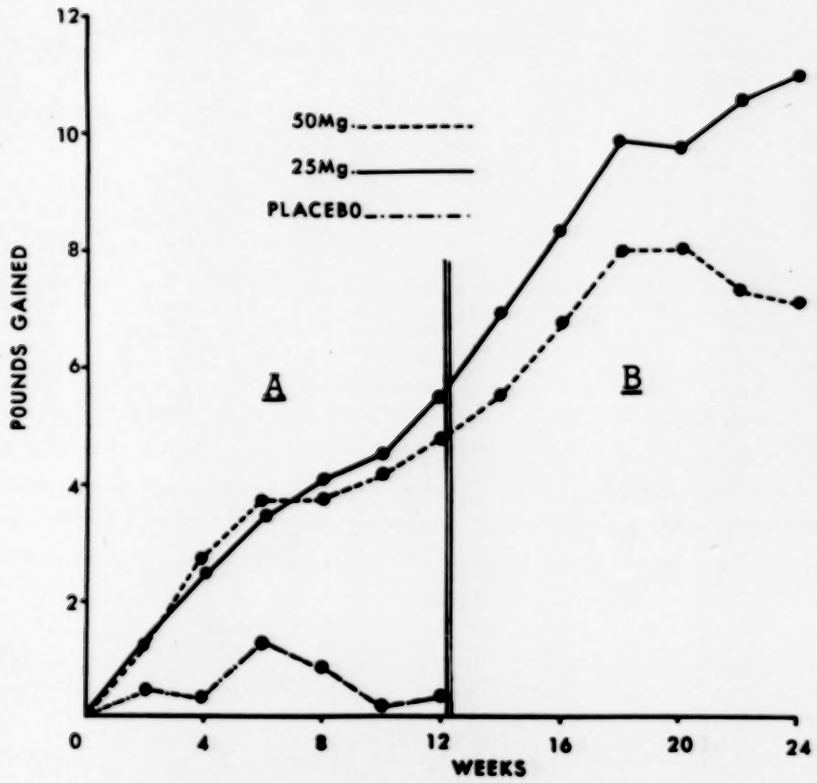

FIG. 1. Mean weight gain of all subjects for twenty-four weeks. A, initial twelve-week double-blind study comparing the effect of placebo, 25 mg. and 50 mg. daily dosages of norethandrolone. Eighteen subjects received the placebo, twenty-three subjects the 25 mg. dosage, and thirteen subjects the 50 mg. dosage. B, effect of continued 25 mg. and 50 mg. daily dosages of norethandrolone for an additional twelve weeks.

istered to the fifty-four subjects by means of the double-blind technic. Prior to giving the medication the patients were weighed on two or more occasions at weekly intervals; these weights were averaged and used as a baseline. Each subject was evaluated weekly for the first month and every two weeks thereafter. All weighings were done on the same set of scales and weights were recorded to the nearest half pound.

Baseline laboratory determinations were made of the differential and total white blood cell counts, hemoglobin, zinc sulfate turbidity, direct and indirect serum bilirubins, and total phospholipids, in addition to chest x-ray and urinalysis. Liver function, lipoprotein and phospholipid studies were repeated at two-month intervals. The remainder of the laboratory studies were repeated when deemed necessary.

Mood changes secondary to the use of the drug were evaluated by several technics. An adjective check list, commonly used in evaluating mood changes, was administered by a clinical psychologist to all subjects in group II initially and again at the end of the first twelve weeks of the study. Subjective ratings of mood were made at the same times. At each biweekly evaluation a second procedure was used to estimate subjective changes in all three groups. The physician asked two questions: (1) "Since taking the medicine, is your appetite the same, better, or worse?" (2) "Since taking the medicine, do you in general feel the same, better, or worse?"

After three months the double-blind technic was terminated and the results evaluated. At that time three subjects in group II were dropped from the study because of illnesses unrelated to the medication. The remaining fifty-one subjects were given either 25 mg. or 50 mg. of norethandrolone daily. At the end of six months a final evaluation was made.

RESULTS

The completion of the double-blind study at twelve weeks made it possible to determine which patients had been receiving norethandrolone and which had been receiving the placebo. The subjects who had received norethandrolone consistently demonstrated weight gains. (Fig. 1A.) The group receiving 25 mg. had gained an average of 5.5 pounds and the group receiving 50 mg. 4.7 pounds. The placebo group showed

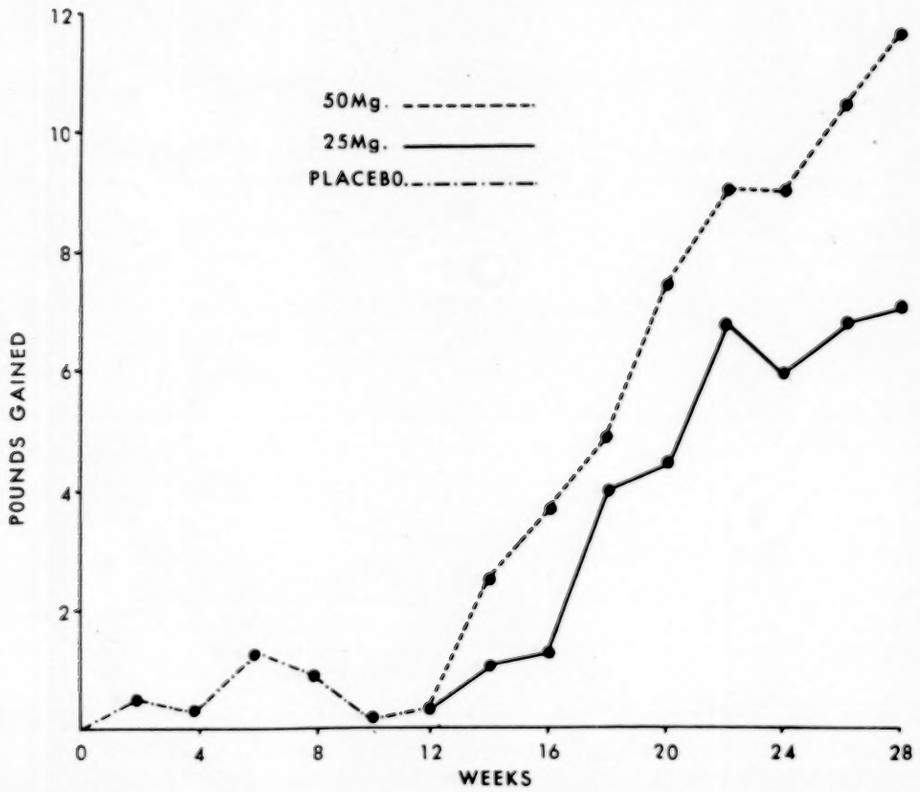

FIG. 2. Mean weight gain in eleven subjects changed from the initial twelve-week placebo administration to 25 mg. (seven subjects) or 50 mg. (four subjects) of norethandrolone daily.

little change over the same period, with an average weight gain of 0.3 pounds. Statistical tests (Table 1) indicate that the weight gains in both norethandrolone groups are significant ($p < .01$) when they are compared to the placebo group.

The total twenty-four–week data reflect the same pattern of weight gain. (Fig. 1B.) Both norethandrolone groups continued to show weight gains, with an average gain of 11.0 pounds for the group receiving 25 mg. and an average gain of 7.1 pounds for the group receiving 50 mg. Although on casual inspection it might appear that 25 mg. was more effective than 50 mg., this was not statistically significant.

TABLE 1
MEAN WEIGHT GAIN FOR FIRST TWELVE WEEKS: NORETHANDROLONE VS. PLACEBO

Drug and Dosage	No. of Subjects	Weight Gain (lb.)
Placebo	18	0.3
Norethandrolone, 25 mg. daily	23	5.5 ($p < .01$)
Norethandrolone, 50 mg. daily	13	4.7 ($p < .01$)

When the placebo group was changed to norethandrolone (Fig. 2) the seven subjects given the 25 mg. dosage gained an average of 5.9 pounds in twelve weeks, and the four subjects given 50 mg. showed an average weight gain of 8.8 pounds in this same twelve-week period. These latter weight gains, although significant ($p < .01$), should not be considered quantitatively precise because of the small number of subjects. The trend is similar, however, to that depicted by the larger series. (Fig. 1.)

The rate of weight gain was consistent for both groups, averaging 0.5 pounds per week, although there was considerable individual variation in the pattern of gain.

Improvement in subjective attitudes was clinically evident to the examiners over the six-month period. The answers to the physician's question about "well-being" reflected this improvement. In the initial twelve weeks those subjects receiving norethandrolone replied that they felt "better" more frequently than those

subjects receiving the placebo. (Table II.) However, the adjective check list carried out on group II showed no clearly defined mood changes.

The improvement in appetite reported by the majority of the subjects receiving norethandrolone was impressive. (Table II.) Many of these subjects developed a voracious appetite while receiving the drug.

Undesirable effects were observed in eight of the ten females. These consisted of menorrhagia in one, acne in four and amenorrhea in seven. These effects were more frequent on the 50 mg. dose and were reversed by reducing the dosage or discontinuing the drug. No hirsutism or changes in voice or libido were observed. Drowsiness, constipation and abdominal distress were observed as commonly in the placebo group as in the norethandrolone group. In one previously asymptomatic young man an acute gastrointestinal hemorrhage developed and he was found to have a duodenal ulcer.

No appreciable changes were noted in the blood counts, serum non-protein nitrogen values, urinalyses or chest x-rays. The effects of norethandrolone on serum lipoprotein, cholesterol and phospholipid levels will be reported separately.

Approximately six weeks after drug administration was started one of the group III subjects, age nineteen, was found to have bromsulphalein (BSP) retention of 41 per cent. Subsequent BSP excretion tests showed abnormal dye retention in the majority of the subjects. The relationship of BSP retention to norethandrolone is the subject of the second paper in this series [*9*].

Twenty-five subjects who had gained weight while receiving the drug were observed for an additional six months after the drug was discontinued. Nineteen either maintained their weight or continued to gain. Of the remaining six subjects the greatest weight loss was 4 pounds except for one man who lost 8 of the 18 pounds gained while receiving norethandrolone. The average weight loss in these six subjects was only 0.7 pounds per month.

TABLE II

APPETITE AND WELL-BEING: RESPONSES OF SUBJECTS TO BIWEEKLY QUESTIONING

Drug and Dosage	Appetite		Well-being	
	Total No. of Responses	No. of Responses Indicating Appetite Was Improved	Total No. of Responses	No. of Responses Indicating Subjects Felt "Better"
Placebo	118	33	132	31
Norethandrolone, 25 mg. daily	122	65	120	45
Norethandrolone, 50 mg. daily	111	62	108	45

COMMENTS

The anabolic effects of norethandrolone have been clearly demonstrated by administration to patients with chronic congestive heart failure [*10*], advanced mammary carcinoma [*11*], hyperthyroidism, poliomyelitis and the nephrotic phase of glomerulonephritis [*12*]. The drug has also been given to a number of patients following major surgery [*7,8*]. In most of these studies norethandrolone was administered to only a few patients with considerable variation in the dosage schedule and duration of therapy.

The subjects in the present study were selected in part on the basis of their freedom from debilitating disease. The weight gains observed in this study were particularly impressive when it is considered that many of the subjects had tried to gain weight for years with vitamins, tonics and patent remedies, without success. It should be noted that eight of the fifty-four subjects did not gain weight while receiving norethandrolone. There was no apparent relationship between the amount of weight gain and age, sex or occupation.

It should be emphasized that the gain in weight resulting from norethandrolone is due to an increase in appetite as well as to the previously reported specific anabolic effect. Although many of the subjects reported an increase in appetite, several who denied any appetite increase showed comparable gains in weight. Previous short term studies have demonstrated an unaltered sodium balance during the administration of norethandrolone [*5,8,12*]. That norethandrolone does not result in excessive sodium retention is supported by the fact that no edema was encountered and no precipitous weight losses occurred after stopping the drug. Of considerable importance was the fact that most subjects maintained their weight gains for at least six months after norethandrolone was discontinued, and a few subjects even continued to gain weight. These findings are entirely in accord with the nitrogen balance data reported in short-term studies [*3,4,5,7,8,11,12*] and support the concept that the increase in weight represents increased protein tissue mass. The important therapeutic implication here is that norethandrolone may be given to induce weight gain with some assurance

that the weight will not be lost soon after the drug is discontinued.

Since the 25 mg. dose appeared to be just as effective as the 50 mg. dose in inducing weight gain, it appeared that even smaller doses might be effective and would result in a lowered incidence of undesirable effects. On this basis three members of our hospital staff were started on a regimen of 20 mg. of norethandrolone daily and have shown impressive weight gains (an average of 12 pounds in two months) with no undesirable effects. Further investigation of smaller dosage schedules is indicated.

Androgenic effects such as hirsutism and voice changes were not observed in this study, although acne did infrequently occur. Amenorrhea occurred frequently, particularly with the daily dose of 50 mg. The mechanism responsible for the amenorrhea is unknown. It is possible that this represents a progesterone-like effect similar to the effect observed previously in the endometrium of the rat [*13*]. Whether the appearance of a bleeding peptic ulcer in the one young male subject was related to norethandrolone administration or was merely a coincidence cannot be determined. The only gastrointestinal symptom noted by any of the other subjects was the increase in appetite.

SUMMARY

1. The clinical effects of a new steroid, norethandrolone (Nilevar), were evaluated in fifty-four chronically underweight persons over a twelve-week period. The "double-blind" technic was employed in the evaluation.

2. Significant weight gain occurred in the groups receiving dosages of 25 mg. and 50 mg. of the drug but did not occur in the group receiving a placebo.

3. Fifty-one of the subjects showed a mean total weight gain of 9 pounds during a six-month period. The weight gain in the groups receiving 25 mg. and 50 mg. did not differ appreciably.

4. The principal undesirable effects were seen in females and consisted of amenorrhea, menorrhagia and acne, all of which were reversed by lowering the dosage or stopping the drug.

5. Twenty-five subjects were observed for six months after the drug was discontinued and nineteen (76 per cent) either maintained their weight or continued to gain.

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A Six-Month Evaluation of an Anabolic Drug, Norethandrolone, in Underweight Persons*

II. Bromsulphalein (BSP) Retention and Liver Function

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In the course of investigating the effectiveness of the anabolic steroid, norethandrolone (Nilevar), in inducing weight gain in essentially healthy persons [*1*], abnormal retention of bromsulphalein (BSP) was inadvertently encountered in one subject. After approximately six weeks of a daily dose of 50 mg. of norethandrolone this subject, a nineteen year old male, showed a BSP retention of 41 per cent. All other liver function tests were normal. Three weeks after norethandrolone was discontinued the BSP test returned to normal. When abnormal BSP retention was found in additional cases, a detailed investigation was undertaken to determine the relationship between norethandrolone and liver function. In addition to careful clinical and laboratory studies on the subjects already receiving the drug [*1*], a short term study in another group of ten patients was designed to delineate more precisely the sequence of BSP changes as well as changes in the serum glutamic oxalacetic transaminase. Kayden [*2*] has recently described elevations of serum bilirubin after administration of norethandrolone to a number of chronically ill patients and has confirmed our observation [*3*] that abnormal BSP retention occurs frequently after administration of the drug.

MATERIAL AND METHODS

Two groups of subjects were used to evaluate the relationship between BSP levels and the administration of norethandrolone.

Long Term Study. The subjects were forty-seven members of the previously reported study of the effect of norethandrolone upon weight gain [*1*]. These subjects received their first BSP test (5 mg. of dye per kg. of body weight) after two months, their second BSP test after four months, and their third test after six months of therapy. Since the period of study had arbitrarily been set at six months, norethandrolone was discontinued at this time. BSP tests were continued at weekly intervals for each subject until the value returned to normal. Serum bilirubin (one-minute and total), alkaline phosphatase, total cholesterol determinations and zinc sulfate turbidity tests were made initially and bimonthly throughout the six-month period of drug administration.

Short Term Study. The subjects for the short term study were ten male geriatric hospital patients with various degenerative disorders but no known liver disease. The average age was sixty-seven years, with a range of forty-three to seventy-seven. Five patients received 25 mg. of norethandrolone daily and the other five 50 mg. daily. BSP tests were performed prior to drug administration and weekly thereafter. The 25 mg. dosage was continued for six weeks but the 50 mg. dosage was given for only three weeks because abnormal BSP retention was well established by that time. BSP tests were continued after norethandrolone was stopped until the BSP values returned to normal. Serum glutamic oxalacetic transaminase determinations were performed by the method of Karmen [*4*] after three weeks of the 25 mg. dosage and after one week of the 50 mg. dosage of norethandrolone. These were repeated at intervals during and after drug administration until they returned to normal.

* From the Research Service, Wood Veterans Administration Center and the Department of Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin. Aided in part by a grant from G. D. Searle and Company, who also supplied the drug as Nilevar.®

TABLE I
RELATIONSHIP BETWEEN MAXIMUM BSP RETENTION AND DOSAGE OF NORETHANDROLONE

Dosage Groups	Total No. of Subjects	BSP Retention		
		0–6%	7–10%	11%+
Subjects receiving 25 mg. of norethandrolone daily	20	9	5	6
Subjects receiving 50 mg. of norethandrolone daily	27	3	4	20

RESULTS

Long Term Study. BSP retention of greater than 6 per cent (upper limit of normal) was found in thirty-five (74 per cent) of the forty-seven subjects tested. Twenty-six (55 per cent) of the forty-seven subjects showed BSP retention in excess of 10 per cent. Eleven persons (23 per cent of the entire group) had dye retention exceeding 20 per cent. There was a clear relationship between the dose of norethandrolone and the degree of BSP retention. (Table I.)

In only two subjects were abnormal BSP levels encountered more than three weeks after stopping the drug. One of these had attained a BSP retention of 57 per cent after receiving 50 mg. of norethandrolone daily for two months. In spite of continued administration of 50 mg. daily the BSP retention fell to 21 per cent at four months and 23 per cent after six months of therapy. Other liver function tests remained normal in this subject until the six-month evaluation when his direct serum bilirubin was 1.0 mg. per cent, his total bilirubin 2.2 mg. per cent, and his serum alkaline phosphatase 7.7 Bodansky units.* The serum proteins, thymol and zinc sulfate turbidities, prothrombin time and esterification of cholesterol all were normal. Liver biopsy at this time showed slight infiltration of lymphocytes into the portal areas, minimal focal necrosis and minimal bile stasis. Both the bilirubin and alkaline phosphatase returned to the normal range within one month after the drug was discontinued, at which time the BSP retention was 19 per cent. This subject's BSP returned to normal six weeks later.

The other subject with persistent BSP retention had a long history of alcoholism. In this patient BSP retention reached 41 per cent after administration of 50 mg. of norethandrolone daily for six months. The direct serum bilirubin was 0.8 mg. per cent, the total bilirubin 1.7 mg. per cent and the alkaline phosphatase 6.0 Bodansky units. Liver biopsy showed areas of marked nuclear activity and moderate infiltration of lymphocytes into the portal areas. Two months after norethandrolone was discontinued his BSP retention was 46 per cent, the direct serum bilirubin 0.5 mg. per cent, total bilirubin 1.2 mg. per cent, and serum alkaline phosphatase was 5.6 Bodansky units. Serum albumin and globulin, cholesterol and esters, glutamic oxalacetic transaminase, prothrombin time, and thymol and zinc sulfate turbidities were normal in this subject. Although pre-existing liver disease had not been diagnosed it is possible that some impairment of liver function may have antedated the administration of norethandrolone. Unfortunately, there was no record of BSP tests in this man prior to this study.

In the remaining forty-five subjects serum bilirubins, serum alkaline phosphatase, total serum cholesterol and serum proteins were normal throughout the study. In a few isolated instances there were elevations of the zinc sulfate turbidity but these were transient and unrelated to BSP retention. All subjects were examined at biweekly intervals for clinical evidence of liver dysfunction. No jaundice, hepatomegaly, liver tenderness, anorexia or weakness could be detected in any of the subjects.

In seven subjects with BSP retention ranging from 14 per cent to 46 per cent, liver biopsies were performed. In four of the biopsy specimens there was slight to moderate infiltration of lymphocytes into the portal areas. Two of these showed areas of "nuclear activity" of the liver cells and one showed minimal focal necrosis and bile stasis. In the remaining three biopsy specimens the histologic appearance was normal.

Short Term Study. The serial values for both BSP and serum glutamic oxalacetic transaminase (SGOT) are shown in Table II.

In the group receiving the 25 mg. dosage abnormal BSP retention was observed in one patient by the end of the first week and in all five patients within four weeks. Minimal elevations of SGOT were observed in only two of the

* Upper limit of normal for these tests are: direct (one-minute) serum bilirubin, 0.25 mg. per cent; total serum bilirubin, 1.0 mg. per cent; alkaline phosphatase, 5 Bodansky units.

TABLE II

SHORT TERM STUDY OF BROMSULPHALEIN (BSP) RETENTION AND SERUM GLUTAMIC OXALACETIC TRANSAMINASE (SGOT)

25 mg. Norethandrolone Daily

Patient	Age (yr.)	Control BSP Retention (%)	BSP Retention and SGOT*							
			Weeks during Norethandrolone Administration						Weeks after Drug Was Discontinued	
			One	Two	Three	Four	Five	Six	One	Two
L. I.	43	3*	5	9	15 (34)	18	16	17 (20)	4.5 (28)	2.5
T. H.	75	4.5	8	16	16 (42)	12	12 (48)	16 (30)	6 (18)	4.5
R. Y.	77	3	3	6	7 (24)	8	15 (26)	19 (30)	5 (14)	4
R. E.	75	3	2.5	3	4 (30)	12	8 (28)	14 (18)	4 (20)	4
K. L.	65	5	13	18	20 (34)	28	7 (36)	40 (44)	9 (30)	4.5
Average	67	3.7	6.0	10.4	12.4 (35)	15.6	12 (34.5)	21.2 (28.4)	5.7 (22)	3.9

50 mg. Norethandrolone Daily

Patient	Age (yr.)	Control BSP Retention (%)	BSP Retention and SGOT						
			Weeks during Norethandrolone Administration			Weeks after Drug Was Discontinued			
			One	Two	Three	One	Two	Three	Four
N. F.	70	4	30 (108)	40	41 (122)	38.5 (104)	23 (56)	10	6
J. C.	66	2.5	8 (16)	18	19 (68)	16 (40)	9 (22)	..	..
S. B.	66	4	11 (20)	9	17 (78)	18 (38)	12.5 (28)	7	..
E. B.	68	3	9 (40)	10	5 (52)	.....	4.5 (24)	3	..
S. H.	69	3	6 (22)	4.5	5 (30)	4 (20)	2.5 (20)	4	..
Average	68	3.3	12.8 (41.2)	16.3	17.4 (70)	19.1 (50.5)	10.3 (30)	6	6

* Upper limit of normal for the BSP test = 6 per cent retention, for the SGOT test = 40 units. Figures in parenthesis represent SGOT values in units.

five patients. These elevated BSP and SGOT values returned to normal within two weeks after discontinuing the drug.

On the 50 mg. dosage four of the five patients showed abnormal BSP retention within one week. All four of these patients showed elevations of SGOT, and the magnitude of the elevations correlated with the BSP retention. Return to normal values for both BSP and SGOT occurred between the second and fourth week after discontinuing the drug. The fifth patient (S. H.) showed neither BSP retention nor elevation of SGOT at any time.

COMMENTS

The 74 per cent incidence of abnormal BSP retention with minimal clinical or other laboratory evidence of liver dysfunction is an unusual response to a drug. Impaired liver function has been observed with such drugs as arsphenamine [*5*], thiouracil [*6*], methyl testosterone [*7*], para-aminosalicylic acid [*8*] and, most recently, chlorpromazine [*9,10*]. However, when abnormal BSP retention was observed with these drugs, it was regularly associated with other evidence of liver or biliary tract dysfunction. In the present study elevation of the serum bilirubin and serum alkaline phosphatase was observed in only two cases, and in both cases was mild and transient. Abnormal BSP retention, however, was present in over 74 per cent of the cases and often was unusually high.

BSP combines with the albumin fraction of the serum proteins following injection [*11,12*]. Less than 2 per cent of the dye is excreted in the urine of normal persons, but in patients with hepatobiliary disease 5 per cent to 10 per cent may be excreted in the urine [*12,13*]; when the serum BSP is particularly high, as much as 25 per cent may be so excreted [*14*]. From 3 per cent to 10 per cent of injected BSP has been recovered from the skeletal muscle [*15*] and lesser amounts from the reticuloendothelial system and other organs [*15,16*].

Most of the dye in combination with serum albumin enters the polygonal cells of the liver [*17,18*] where it forms stable complexes with various liver protein fractions [*19*], and after varying periods of storage is excreted into the bile [*15,17,20*].

Several mechanisms, either individually or in combination, may account for BSP retention.

1. Lowered serum albumin with reduction in quantity available for combination with BSP: Since serum proteins were normal throughout the norethandrolone study, this mechanism would not explain the abnormal BSP values.

2. Reduced dye-binding capacity of the serum proteins: Although diminished dye-binding capacity of the serum has been reported in several pathologic states [*21,22*], Blondheim in a recent survey of this problem [*23*] reported a close correlation between the dye-binding capacity and the serum albumin concentration in both normal and diseased subjects except when jaundice supervenes. Nevertheless, a reduction in the dye-binding capacity of serum cannot be excluded as a possible mechanism for the BSP retention produced by norethandrolone.

3. Blockage of urinary excretion of BSP: Since less than 2 per cent of the dye is normally excreted in the urine [*12,13*], complete blockage could not account for this degree of BSP retention.

4. Reduced uptake of BSP by skeletal muscle: It is possible that the anabolic action of norethandrolone could affect skeletal muscle by inhibiting its uptake of the dye. However, since less than 8 per cent of BSP storage is in skeletal muscle [*15*] this mechanism could at best account for only a small fraction of the BSP retention observed with norethandrolone.

5. Reduced hepatic circulation: The possibility that norethandrolone may diminish liver blood flow and consequently hepatic excretion of the dye is unlikely since no effect of the drug on the vascular system has been observed.

6. Bile stasis: Blockage to bile flow causes retention of BSP only when secondary hepatocellular changes ensue, since the liver cells are capable of storing amounts of BSP in excess of the test dose [*15*]. Primary bile stasis would be regularly associated with high serum alkaline phosphatase as well as high serum bilirubins.

7. Failure of transfer of BSP from the blood into the bile: The transfer of BSP from the blood into the bile is believed to consist of three steps [*5,9*]: (*a*) the uptake of the dye by the liver cells from its combination with the circulating serum albumin; (*b*) storage of the dye in the liver cells; and (*c*) the excretion of dye into the biliary tract. Either the uptake or the excretion of the dye may involve an enzyme system [*14*] or some alteration in cell membrane characteristics. Several agents have been shown to inhibit BSP excretion; among these are sodium dehydrocholate [*20*], rose bengal [*11*] and probenecid [*14*]. In all three of these interference with the

transport system from the blood to the bile has been postulated.

It seems most likely that the BSP retention induced by norethandrolone results from interference with the transport mechanism, either the uptake of BSP by the liver cells or the excretion of the dye into the biliary tract.

Although the striking and consistent BSP retention is unusual and out of proportion to the clinical and other laboratory changes, it seems reasonable to conclude that norethandrolone is to some extent hepatotoxic, particularly at high dosage levels. The elevations of serum bilirubin, alkaline phosphatase and glutamic oxalacetic transaminase, although slight and infrequent, support this thesis, as do the abnormalities described in the liver biopsy sections. Gutman, in a recent editorial [*24*], has included norethandrolone in a list of hepatotoxic drugs and suggests that some degree of hepatocellular dysfunction may be present despite the normal histologic appearance of the liver. Recent studies by Schaffner and his associates [*25*] provide further evidence in support of this thesis by their observation of four instances of distinct histologic and biochemical evidence of "cholestasis" in their series of twenty-seven patients receiving 60 mg. of norethandrolone daily.

It is of interest that the weight gain induced by norethandrolone does not correlate with dosage [*1*], whereas the effect of the drug on liver function does appear to be related to dosage. It has been possible to obtain excellent weight responses with minimal or no BSP retention or other evidence of liver dysfunction. In the clinical use of norethandrolone, particularly in long term therapy, a dose of 20 mg. daily should be anabolic in most cases and should lessen the risk of liver toxicity. Tests of liver function are indicated if the drug is to be given for periods of more than one month. The most sensitive tests are the BSP and the serum glutamic oxalacetic transaminase. However, mild increases in BSP retention and the SGOT level are so commonly encountered after norethandrolone that these tests are of little use in assessing serious liver dysfunction. The appearance of an elevated serum alkaline phosphatase or elevated serum bilirubins (one-minute bilirubin in excess of 0.25 mg. per cent or total bilirubin in excess of 1.0 mg. per cent) suggests that the process is more severe.

Patients with antecedent or existing liver disease might well be more sensitive to the hepatotoxic effects of norethandrolone. Although liver disease of itself is not an absolute contraindication to the drug, norethandrolone administration in such patients should require a compelling indication, and the course of the patient should be carefully followed.

On the other hand, the young healthy subjects who participated in this study showed little or no evidence of hepatic dysfunction following administration of norethandrolone. Eight of the subjects in the original six-month study elected to remain on the drug and have received norethandrolone for fourteen to sixteen months. During the last week of this prolonged period of norethandrolone administration, two of the eight subjects showed BSP retention of 45 per cent and 22 per cent. BSP retention in the other six ranged from 2.5 per cent to 12 per cent. The alkaline phosphatase, serum bilirubin and serum glutamic oxalacetic transaminase all were normal and the clinical course in each case was completely satisfactory.

We may conclude that norethandrolone does impair liver function to varying degrees and with varying frequency. However, this impairment appears to be regularly reversible and is less frequent and less severe when lower dosages are used. Since the drug retains its anabolic effectiveness in dosages of 20 mg. daily, we believe that it may be used as an anabolic agent with minimal risk at this lower dosage level.

SUMMARY

1. Abnormal BSP retention was found in 74 per cent of forty-seven subjects who received norethandrolone in order to induce weight gain.

2. Liver function studies were normal in all but two of the subjects who showed slight elevation of serum bilirubins and serum alkaline phosphatase. These returned to normal several weeks after stopping the drug.

3. No clinical evidence of liver dysfunction could be obtained in any of the subjects.

4. Of the seven liver biopsies obtained, four showed minimal abnormalities consisting of increased nuclear activity and slight to moderate infiltration of lymphocytes. One of these showed minimal focal necrosis and minimal bile stasis. The remaining three biopsy specimens had a normal histologic appearance.

5. A short term study in ten geriatric patients indicated that a 25 mg. daily dose would result in abnormalities of BSP and SGOT in two to

three weeks. The 50 mg. daily dose results in similar abnormalities after only one week.

6. The possible mechanisms for the BSP retention are discussed. It is considered probable that norethandrolone inhibits the transfer of BSP from the blood into the bile.

7. The mild and reversible nature of the liver dysfunction suggests that norethandrolone in low dosage may be safely employed as an anabolic agent, even for long term therapy.

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Cholestasis Produced by the Administration of Norethandrolone*

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THE pathogenesis of intrahepatic cholestasis [*1*], often designated as cholangiolitis [*2*], remains problematic. It frequently appears following the administration of various drugs [*3*] such as arsenicals [*4*], chlorpromazine [*5–7*], methimazole [*8*], para-aminosalicylate [*9*] and methyltestosterone [*10,11*].

To try to contribute to the understanding of intrahepatic cholestasis and to call attention to another drug producing it, observations on patients receiving norethandrolone are reported. Norethandrolone (17-alpha ethyl-17-hydroxy norandrosterone, Nilevar®) is a compound with metabolic activity similar to methyltestosterone but with fewer masculinizing effects [*12*]. The drug is used to improve protein nutrition and to cause weight gain in depleted patients.

MATERIAL AND METHODS

Norethandrolone, in doses of 60 mg. a day, was administered from three to five weeks to twenty-seven patients with various chronic diseases. Apart from cholestasis, it produced no definite side reactions when administered orally in doses usually up to 50 mg. a day.

Patients with chronic diseases were selected as subjects of the investigation since they would be the ones to whom the drug would more likely be given, to improve their chronic nutritional depletion. Drugs known to produce cholestasis, such as chlorpromazine, were not given during the period of study. A group of twenty-eight patients receiving a new tranquilizing agent (Dartal®) served as a control series. The patients were examined each day and weighed weekly. Hepatic tests including cephalin flocculation, thymol turbidity, turbidimetric serum gamma globulin, serum and urinary bilirubin, serum alkaline phosphatase, and serum glutamic oxalacetic-transaminase activity (colorimetric) were performed initially and at weekly intervals during the period of administration of the drug. Liver biopsies were performed with a Terry needle using a transthoracic approach. The biopsy specimens were fixed in buffered formalin and stained with hematoxylin and eosin. Special stains such as fat and iron stains were used when indicated. The tissues were examined without knowledge of the patient's status. Clinical observations were made without knowledge of the results of either the biopsy or the hepatic tests.

RESULTS

In almost all biopsy specimens taken from patients before or after treatment with norethandrolone a slight increase of intralobular and perilobular ductules was noted and the portal tracts revealed a varying increase of cells, primarily histiocytes, lymphocytes, and an occasional eosinophilic leukocyte. Occasional fat droplets and portal fibrosis were ignored.

Non-specific reactive hepatitis, as defined by few areas of focal necrosis, focal or diffuse proliferation of Kupffer cells and slight to moderate inflammatory infiltration in the portal tracts usually associated with ductular proliferation [*16*], was found before and after administration of norethandrolone, without significant influence by the drug. (Table 1.) The same holds true for conspicuous portal inflammation which was characterized by accumulation of lymphocytes, histiocytes, neutrophilic and sometimes eosinophilic segmented leukocytes in varying proportions in and around the portal tract and frequently also around proliferated intralobular and perilobular ductules ("cholangioles"). This picture is frequently designated cholangiolitis and is sometimes associated with portal edema. Cholestasis, as defined by bile pigment in the liver cells and Kupffer cells and feathery degeneration of liver cell cytoplasm [*16*], appeared in four patients after the administration of norethandrolone. In one instance this was asso-

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TABLE I

CLINICAL AND LABORATORY DATA IN PATIENTS IN WHOM CHOLESTASIS WAS FOUND AFTER ADMINISTRATION OF NORETHANDROLONE

Case and History	Week	Transaminase SGO-T (units)	Alkaline Phosphatase (Bodansky units)	Serum Bilirubin (mg. %)	Urinary Bilirubin	Cephalin Flocculation	Thymol Turbidity (units)	Turbidimetric Gamma Globulin (gm. %)
CASE I. 54 year old man with right hemiparesis following excision of meningioma in 1953; physical examination showed hemiparesis but no other abnormalities; norethandrolone administration was discontinued after second week when jaundice appeared; initial specimen showed mild non-specific reactive hepatitis; later specimen showed cholestasis but other abnormalities were less marked	0	12	4.4	0.7	0	0	0.6	1.10
	1	216	6.4	1.9	4+	0	0.8	0.97
	2	224	7.1	8.4	4+	0	0.8	0.86
	3	150	10.2	15.0	4+	0	0.5	0.77
	4	140	48.2	32.0	4+	0	2.4	1.25
	5	95	79.0	8.6	4+	0	0.5	1.33
	6	70	66.1	8.3	3+	0	0.5	0.69
CASE II. 63 year old man with rheumatoid arthritis, inactive of the left hip, shoulder and elbow, but with limited motion; after four weeks of norethandrolone, gained 3 pounds and felt better; patient was transiently icteric in final week of study; liver normal initially, and showed only cholestasis later	0	7	1.3	0.5	0	0	3.2	1.40
	1	78	1.0	0.5	0	2+	1.9	1.06
	2	80	1.0	0.4	0	0	1.0	1.20
	3	100	2.0	0.4	0	0	1.5	1.10
	4	180	1.5	1.8	0	0	0.5	0.80
	5	126	2.5	0.8	1+	0	0.8	0.86
CASE III. 54 year old man with right hemiplegia one year previously and arteriosclerotic heart disease; atrial fibrillation and heart failure developed which responded to digitalis while under observation; patient was mildly icteric in last week of study; liver normal initially and showed portal infiltration and ductular proliferation later	0	10	2.0	0.2	0	1+	1.5	1.30
	1	38	2.1	0.3	0	1+	2.1	0.89
	2	24	2.0	0.5	0	0	0.6	1.00
	3	40	2.4	0.4	0	0	2.0	1.10
	4	60	2.8	1.0	0	0	2.5	0.65
	5	195	4.7	0.8	1+	2+	0.8	0.72
	6	38	3.6	1.4	0	0	1.9	0.86
CASE IV. 68 year old man with hypertensive and arteriosclerotic heart disease with some hypertensive encephalopathy for about two years; no change of norethandrolone except for 6 pound weight gain; liver normal initially and showed only cholestasis later	0	14	3.4	0.5	0	0	1.0	1.50
	1	190	4.6	0.7	0	0	1.5	1.30
	2	190	6.3	0.7	0	0	1.5	1.30
	3	37	3.9	0.4	0	0	2.4	1.04
	4	50	2.9	0.3	0	0	1.9	1.04

ciated with a moderate degree of portal inflammatory reaction and also some areas of intraparenchymal focal necrosis, not present in the initial biopsy specimen. In two instances the picture of the liver had not changed except for the appearance of cholestasis. (Fig. 1.) In a fourth instance a non-specific reactive hepatitis with moderate portal inflammation and edema found in the initial biopsy specimen was absent in the specimen obtained after norethandrolone treatment. (Fig. 2.) This was the patient with the greatest degree of jaundice, which developed after two weeks of norethandrolone administration, when therapy was stopped. The serum alkaline phosphatase and transaminase activities rose precipitously. (Table II.) The patient remained jaundiced for ten weeks. The second liver biopsy was performed four weeks after norethandrolone therapy was discontinued (the prothrombin time was too prolonged for earlier examination). The results of the other hepatic tests remained normal. Three months after the second biopsy the results of all the tests were normal except the serum alkaline phosphatase which was slowly declining. Clinically, aside from some anorexia and malaise initially, the patient had no symptoms.

Of the remaining twenty-three patients, thirteen, who showed some degree of non-specific reactive hepatitis or portal inflammation initially, exhibited no essential change in the biopsy specimens after administration of norethandrolone for three to four weeks. (Table II.) In one patient only slight ductular proliferation and portal inflammation seen in the first biopsy specimen was fairly severe in the second one.

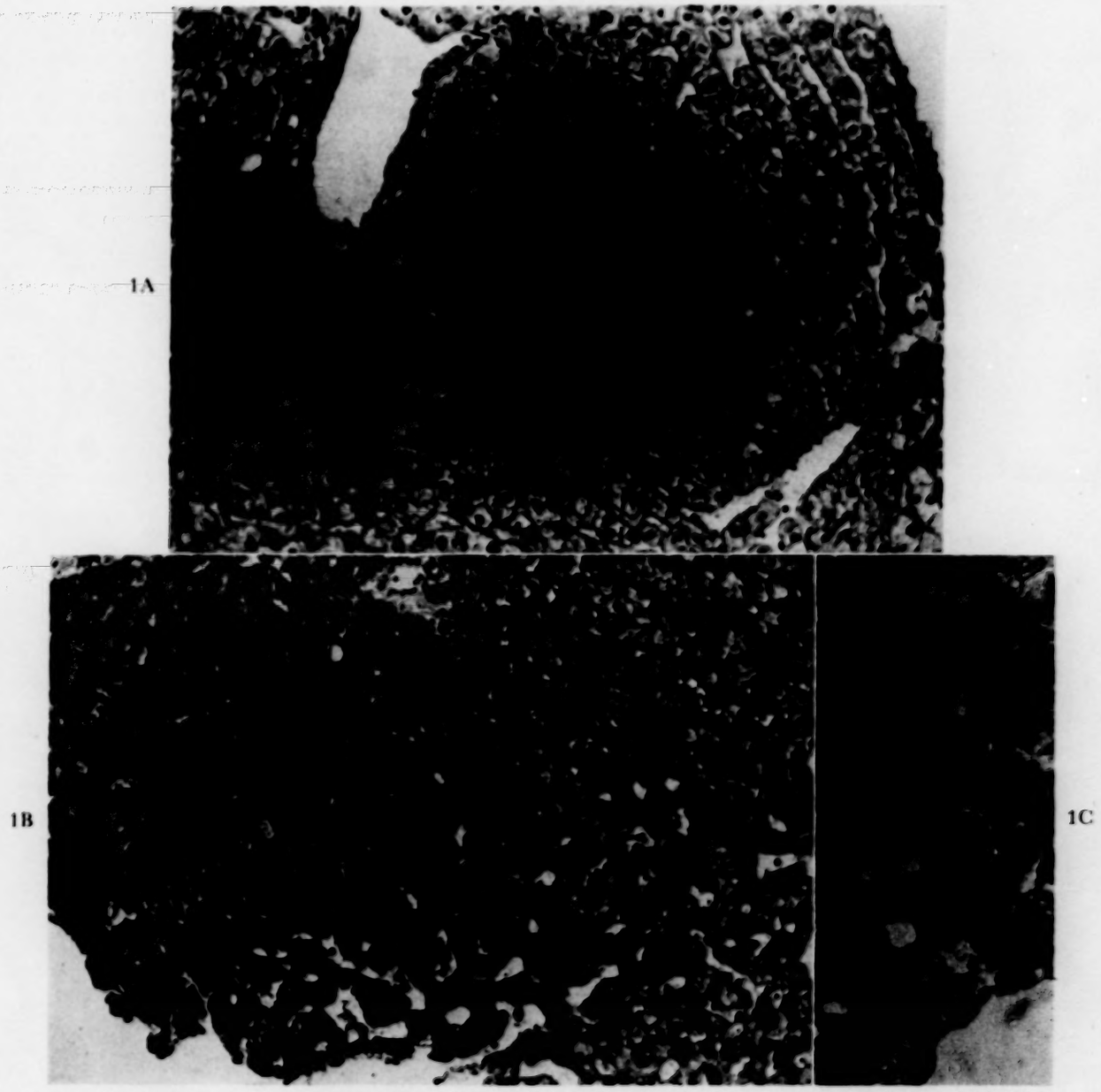

FIG. 1. A, essentially normal liver before administration of norethandrolone. Hematoxylin and eosin × 80. B, biopsy specimen after administration of norethandrolone, unchanged except for the presence of cholestasis. Hemotoxylin and eosin × 80. C, higher magnification showing large bile plug (arrow) in canaliculus. Hematoxylin and eosin × 400.

In another patient the reverse occurred. The serum glutamic oxalacetic–transaminase activity was normal initially in all patients. Abnormally high activity, above 40 units, developed in nine of the patients in this group, the highest value being 140 units. (Table III.) The serum alkaline phosphatase activity did not increase in any patient. Six of the thirteen patients had abnormally high gamma globulin levels at the onset but not at the end of the period of observation. No other abnormalities were noted in any of the other hepatic tests performed.

In the remaining ten patients, normal or nearly normal liver tissue was obtained before and after the drug was given. (Table II.) In three of the patients serum glutamic oxalacetic–transaminase activity increased to above 40 units, the highest value being 64 units, while in the remaining seven patients a mild elevation within the normal range from the initial values

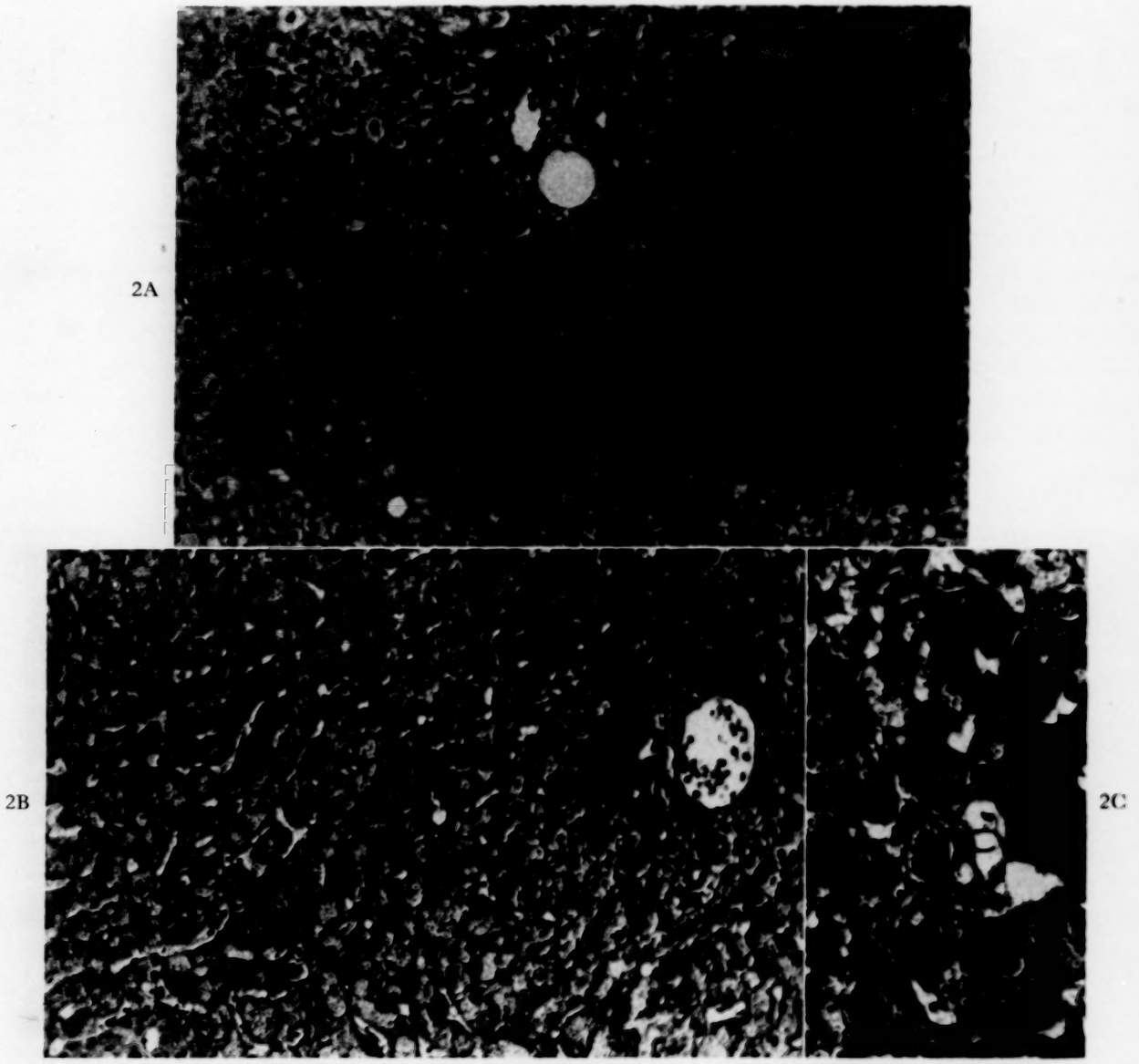

FIG. 2. A, non-specific reactive hepatitis with moderate portal inflammation before administration of norethandrolone. Hematoxylin and eosin × 80. B, specimen obtained four weeks after onset of jaundice showing cholestasis but less portal inflammation. Hematoxylin and eosin × 80. C, higher magnification showing bile plugs (arrows) in bile capillaries. Hematoxylin and eosin × 160.

was noted. Aside from isolated abnormally high cephalin flocculation and gamma globulin, the results of the remainder of the tests were normal.

All but two of the entire group of twenty-seven patients gained weight, ranging from 1 to 9 pounds, while receiving norethandrolone.

Cholestasis did not develop in any of the twenty-eight patients in the control series receiving the tranquilizing, nor were any changes observed in the results of the various hepatic tests. The large doses of this drug which were used produced rather severe anorexia and malaise which in turn led to increased malnutrition and dehydration. This was held responsible for some histologic evidence of hepatic damage which was not reflected clinically or in the results of the hepatic tests.

COMMENTS

Cholestasis has been defined as the phenomenon produced by interference with bile flow after its formation by the liver cells [*13*]. This term has been confused with cholangiolitis, which implies inflammation around the smallest

TABLE II

DISTRIBUTION OF PATIENTS WITH AND WITHOUT CHOLESTASIS ACCORDING TO THE HISTOLOGIC FINDINGS IN THE LIVER BIOPSY SPECIMEN BEFORE AND AFTER ADMINISTRATION OF NORETHANDROLONE

	Normal	Non-specific Reactive Hepatitis			"Cholangiolitis"		
	Before and After	Before and After	Before Only	After Only	Before and After	Before Only	After Only
No cholestasis	10	4	2	1	4	1	1
Cholestasis	2*	..	1	..	..	..	1

* Except for cholestasis.

intralobular and periportal bile ducts or ductules. Cholangiolitis is often associated with cholestasis, and the two terms have been used synonymously although the entities can be separated from each other histologically.

Cholestasis has usually been ascribed to viral hepatitis but recently various drugs such as chlorpromazine seem more frequently to be responsible. The livers of patients with chlorpromazine jaundice show inflammatory reactions characterized by mononuclear and often eosinophilic infiltration around the small bile ductules and in the portal tracts [*5,6*]. However, in cholestasis induced by arsenicals [*4*] or methyltestosterone [*10,11*] this inflammatory reaction is absent or minimal. This is true also of early chlorpromazine jaundice. Most instances of cholestasis are associated with increased serum alkaline phosphatase activity and increased serum and urinary bilirubin, whether the interference with the bile flow is caused by viral hepatitis or by drugs such as chlorpromazine [*7*]. In all cases in which cholestasis was found after administration of norethandrolone, serum glutamic oxalacetic-transaminase activities were considerably higher when it was absent. These high levels preceded the development of jaundice. The rise in serum alkaline phosphatase activity lagged behind that of the transaminase and in some instances failed to appear altogether. Decreasing serum and urinary bilirubin values also lagged behind the rise in transaminase and failed to increase in one instance. The absence of inflammation of any significant degree around the bile ductules after administration of norethandrolone and the lack of correlation with the biochemical findings suggest that this form of cholestasis is neither inflamma-

TABLE III

SERUM GLUTAMIC OXALACETIC TRANSAMINASE (SGO-T) ACTIVITY (IN UNITS) AND TUBIDIMETRIC GAMMA GLOBULIN (IN GRAMS) OF PATIENTS TAKING NORETHANDROLONE

Data	No. of Patients	Test	Weeks on Norethandrolone					
			0	1	2	3	4	5
Patients with definite cholestasis after norethandrolone therapy	4	SGO-T	11	130	130	82	108	104
		Gamma globulin	1.32	0.98	1.09	1.00	0.93	0.97
Patients without cholestasis but with nonspecific hepatic changes or "cholangiolitis" before or after norethandrolone therapy	13	SGO-T	12	33	56	68	68	29
		Gamma globulin	1.35	1.25	1.16	0.98	1.06	1.11
Patients with normal or almost normal hepatic structure before and after norethandrolone therapy	10	SGO-T	10	31	25	21	21	....
		Gamma globulin	1.08	0.95	0.96	0.94	1.00	....

tory nor allergic in the usual sense of the word. Perhaps norethandrolone may alter the permeability of the bile ductules in some patients or may increase the viscosity of the bile by injuring the liver cells which secrete it.

Inflammatory changes in and around portal tracts and non-specific changes in the hepatic parenchyma were common findings in biopsy specimens even before any drugs were given. In most instances these findings persisted unchanged and were not consistently associated with any biochemical alteration. The changes probably result from aging, previous illnesses and inadequate nutrition. They have been seen in livers of normal persons dying suddenly but to a less marked degree [*13*] and apparently are in no way related to cholestasis.

In this series, in four of twenty-seven patients receiving norethandrolone histologic evidence of cholestasis developed with some biochemical abnormalities and clinical signs. This did not occur in a control series of patients given a tranquilizing drug. Jaundice may occur after only two weeks of administration of norethandrolone and may be severe. On the other hand, it may appear late and in a quite transient and mild form. The presence of pre-existing hepatic damage or of another disease does not appear to be a factor in the development of the cholestasis, although it may determine its severity. The degree of cholestasis seen histologically cannot be well correlated with either clinical or laboratory features. However, serum transaminase activities above 150 units or elevation of the serum bilirubin level indicates that the drug should no longer be given.

The administration of norethandrolone results in a significant gain in weight in most patients, owing to the effect of the drug on protein metabolism. Some of the increase of the serum glutamic oxalacetic–transaminase activity may be an indication of the augmented protein anabolism.

SUMMARY AND CONCLUSIONS

Norethandrolone was administered to twenty-seven patients for three to five weeks. Liver biopsies were performed before and after administration of the drug. Histologic evidence of cholestasis was found in four patients. In each of these instances the serum glutamic oxalacetic–transaminase activity increased above 150 units from normal levels. In one patient severe jaundice developed which lasted ten weeks. The remaining patients tolerated the drug well and gained weight. In a control group of twenty-eight patients receiving a tranquilizing drug and similarly studied no instances of cholestasis were found.

Inflammatory reaction in the portal tracts and around proliferated ductules (cholangiolitis) was not associated with the cholestasis although it was present in some patients before and after administration of norethandrolone.

Acknowledgment: We wish to thank Dr. Joseph Musci for his diligent and splendid cooperation in the clinical management of the patients.

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Aortitis and Aortic Regurgitation Associated with Rheumatoid Spondylitis*

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THE association of heart disease with peripheral rheumatoid arthritis has been accepted for a number of years [*1–5*]. More recently, a characteristic clinical and pathological lesion of the aorta and aortic valve associated with rheumatoid spondylitis has attracted attention [*6–19*]. The chief clinical characteristic has been an aortic regurgitation, but the lesion has been manifested at times by cardiac enlargement, conduction changes, pericarditis, substernal pain and congestive heart failure [*8–11,13–15,17–19*]. Pathological changes have been located chiefly in the root of the aorta, and have been characterized by focal destruction of the elastica in the media, endarteritis of the tunica externa, the development of pale, non-calcified plaques in the intima, and rolling of the aortic valve margins without fusion of the cusps [*6,10,14,16,19*].

Speculation as to the cause and nature of this lesion has varied but generally has assumed the following trends: (1) a lesion due to rheumatic fever which is, or has been, present along with rheumatoid spondylitis; (2) a variation of the cardiac lesions associated with peripheral rheumatoid arthritis; (3) a lesion due to syphilis; or, (4) a lesion specifically related to the rheumatoid spondylitis.

Bernstein [*11,13*] was one of the first to propose that the cardiac and aortic disease associated with rheumatoid spondylitis was due to rheumatic fever. She found, in the course of examining 352 patients with rheumatoid spondylitis, that ten had some type of valvular heart disease. Eight patients gave a history suggesting rheumatic fever, and aortic insufficiency was found in six of these eight. The report of Blumberg and Ragan [*17*] gave support to this view. In a review of 128 patients with rheumatoid spondylitis, valvular disease was detected in six patients of whom four had aortic insufficiency. Four of the six patients gave a history of having had rheumatic fever. On the other hand, Clark [*10,14,19*] and Schilder [*18*] disagreed. Furthermore, pathological reports showed that the microscopic changes in the aorta and aortic valve were distinctly different from those observed in rheumatic fever [*6,10,14,16,19*].

The second opinion, that this type of aortitis and aortic valve disease is a variation of the lesions seen in peripheral rheumatoid arthritis, seems reasonable in view of the apparent close relationship between the two diseases. Observations made by a number of workers [*1–5*] fail to reveal changes in the root of the aorta and the aortic valve such as have been described in the type of cases under consideration. For the greater part, pathological changes in the heart associated with peripheral rheumatoid arthritis have consisted of pericardial, myocardial and mitral valve lesions. Furthermore, Schilder [*18*] states that in the examination of several hundred cases of mitral valve disease, a lesion most likely to be associated with rheumatic fever or peripheral rheumatoid arthritis, not a single case of rheumatoid spondylitis was encountered. In this series of five cases [*18*] of aortitis and rheumatoid spondylitis, only one patient had peripheral rheumatoid arthritis. In a series of twenty-two cases Clark [*19*] reported on three patients who had no peripheral joint disease. In the other cases reported, rheumatoid spondylitis was present in all, with or without peripheral rheumatoid arthritis [*8,11,13,15,17*].

Syphilis bears consideration as a possible cause in any case of aortic regurgitation. This relationship has been strengthened by the nature of the pathological lesion, which closely resembles the changes seen in syphilitic aortitis; in the past it has been mistaken frequently for this lesion. Clark [*19*] excluded patients who had syphilis

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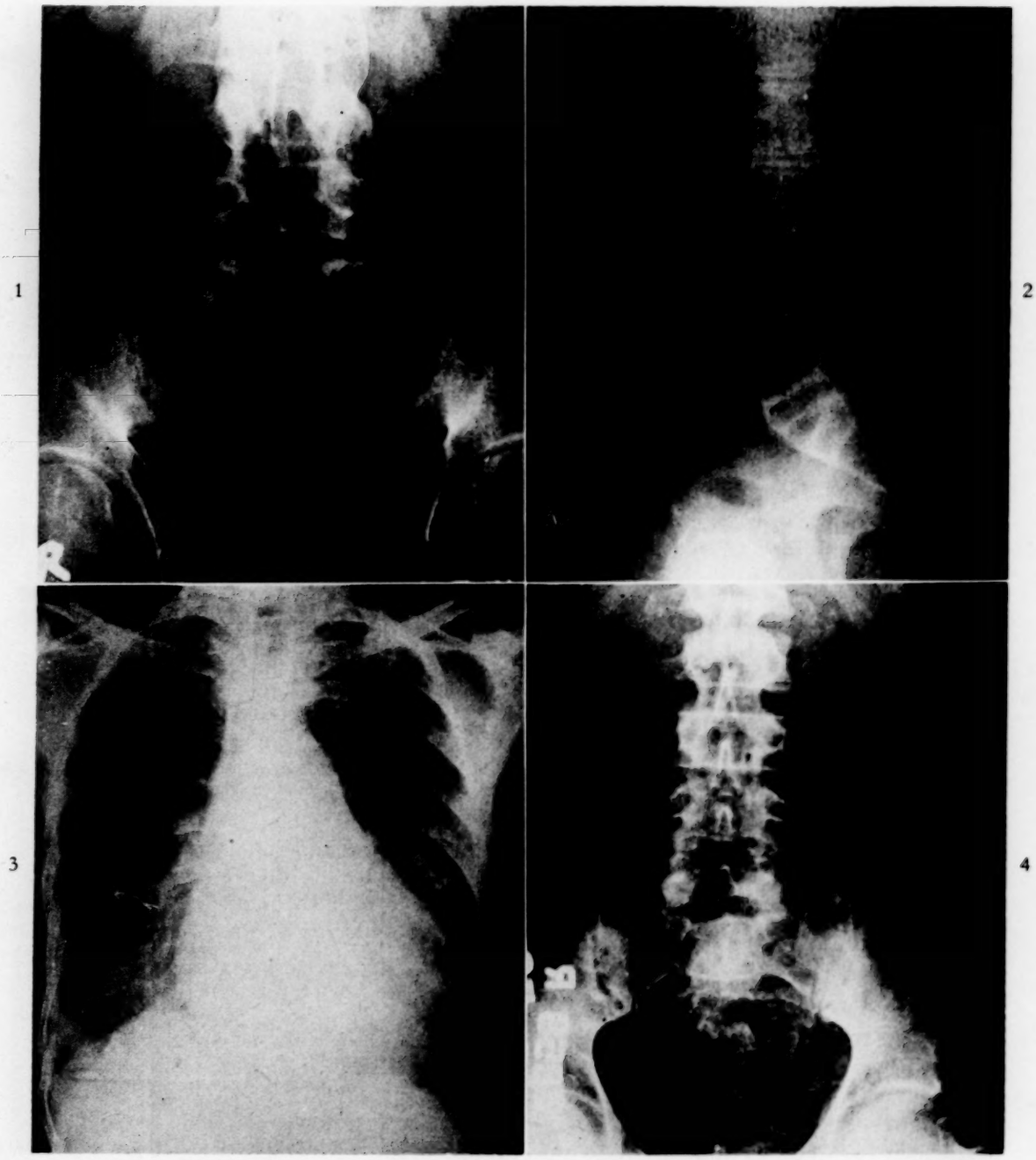

Fig. 1. Case VII. Ferguson view of sacroiliac joint showing narrowing, irregular ragged margins with areas of bone production and destruction.

Fig. 2. Case VI. Roentgenogram of the lumbar spine. There is calcification of the anterior spinal ligament and loss of curvature.

Fig. 3. Case I. Roentgenogram of the chest. There is general cardiac and left ventricular enlargement, moderate pulmonary congestion, and a small amount of fluid in the right pleural cavity. (Figs. 3 and 4 from: Toone, E. C., Jr., Pierce, E. L. and Hennigar, G. R. Aortic insufficiency and rheumatoid spondylitis. In: Progress in Arthritis, pp. 154–176. Edited by Talbot, J. H. New York, 1958. Grune & Stratton, Inc.)

Fig. 4. Case I. Roentgenogram of the pelvis. The sacroiliac joints are irregular and narrowed and show areas of bone production and bone destruction.

from a series of twenty-two cases, four of whom had negative TPI (Treponema pallidum immobilization) tests. Schilder [*18*] found negative histories and STS (serologic test for syphilis) reactions in a series of five cases. In other reports [*10,11,13,14*] the STS reactions were negative and clinical manifestations of syphilis were absent.

On the other hand, evidence is increasing to support the opinion that the type of aortitis and aortic regurgitation in question is specifically related to rheumatoid spondylitis [*10,14,18,19*]. It is the purpose of this report to attempt to strengthen this opinion by describing eight additional cases of aortic regurgitation and aortitis noted in the study of 265 cases of rheumatoid spondylitis, two with postmortem examinations. The diagnosis of rheumatoid spondylitis was made on the basis of typical x-ray changes in the sacroiliac joints, and characteristic physical findings. (Figs. 1 and 2.) The diagnosis of aortic regurgitation was made on the basis of a diastolic murmur heard over the aortic valve and along the left sternal border by at least two observers.

CASE 1. R. E. H., a fifty-four year old Negro man, was admitted to St. Philip Hospital in July 1956 complaining of exertional dyspnea and orthopnea of two weeks' duration, associated with pain and stiffness in the low back, knees, shoulders, wrists and hands. Treatment consisted of the use of digitalis, mercurial diuretics and x-ray therapy to the lumbosacral spine, with satisfactory improvement and discharge after two weeks. The patient was readmitted in September 1956 with acute pulmonary edema which resulted from the voluntary discontinuance of digitalis. Response to the reinstitution of treatment was prompt and satisfactory, and the patient was discharged after one week. The final admission occurred in December 1956 because of severe shortness of breath, evidence of congestive heart failure, and a constant, severe substernal pain. Vigorous treatment for the acute congestive failure was instituted, and nitroglycerin was given for the substernal pain, but without benefit. The patient's condition grew progressively worse, and death occurred five days after admission.

The patient had been hospitalized in February 1953 because of fever, chills, productive cough, weight loss and generalized stiffness and aching in the muscles and joints, of eight weeks' duration. The following diagnoses were made at that time: pericarditis, based on the presence of a friction rub and electrocardiographic changes; first degree heart block; possible rheumatoid arthritis, based on changes in the x-ray films; and abdominal pain of an unexplained nature, leading to an exploratory laparotomy with no abnormal findings. There was a second hospitalization in July 1953 because of malaise, anorexia, pain in the anterior chest, fever, and shortness of breath on exertion. The following diagnoses were made: pneumonitis, lower left lobe; urethritis, non-specific; conjunctivitis (iritis ?); and recurrent pericarditis. In November, 1954, he had again been hospitalized because of a productive cough, dyspnea on exertion, and intermittent swelling of the knees and ankles. The diagnoses were: conjunctivitis; stomatitis; recurrent arthritis, type undetermined; and fever, undetermined origin. There was no history of rheumatic fever or syphilis.

Physical examination in July 1956 revealed the blood pressure to be 140/50 mm. Hg, the pulse rate 100, rhythm regular, and temperature 102°F. The cardiac apex was in the sixth intercostal space in the anterior axillary line. There was a grade 3 high pitched, decrescendo diastolic murmur heard loudest at the third left intercostal space, but present also over the aortic valve area and along the entire left sternal border. A grade 2 soft, blowing systolic murmur was heard at the apex. Rales were present in both lung bases, and the liver was moderately enlarged and tender. The back was limited in its range of motion in all directions, and forward flexion was to within 10 inches of the floor. The chest expansion was restricted to ¾ inch. The wrists were enlarged and limited in their range of motion, both for flexion and extension. Both shoulders were limited in their range for abduction and external rotation, and there was moderate synovial thickening of both knees. Small, firm, freely movable nodules were present on the extensor surfaces of both elbows.

X-ray examination showed a grossly enlarged heart with a hypertensive configuration, and a moderately arteriosclerotic aorta. There was moderate pulmonary congestion, and a small amount of fluid was present in the right pleural cavity. (Fig. 3.) An electrocardiogram showed evidence of left ventricular strain and left bundle branch block. The sacroiliac joints, on roentgen examination, were irregular and narrowed, with areas of bone production and bone destruction. (Fig. 4.) On the anterior margin of the third lumbar vertebra a small area of calcification was noted, suggesting early calcification of the anterior ligament. These changes were interpreted as those of early rheumatoid spondylitis. Roentgenograms of the right wrist and hand showed changes of moderately advanced chronic arthritis (rheumatoid). The STS was negative; the erythrocyte sedimentation rate 51 mm.; the sheep cell agglutination test negative; the antistreptolysin titer less than 50; the L.E. cell preparation negative; the C-reactive protein 2 plus; the total protein 7.4 gm. with albumin of 2.8 gm. and globulin of 4.6 gm.; the hemoglobin 13.5 gm. per cent, and the leukocyte count 6,800 per cu. mm.

On gross pathological examination the aorta was thick-walled and dilated, most prominently in the

FIG. 5. Case 1. Aorta with intima covered by a layer of fibrin. Exuberant foci of inflammation are present in the intima, and smaller foci in the media. (Figs. 5, 6, 7 and 8 from: TOONE, E. C., JR., PIERCE, E. L. and HENNIGAR, G. R. Aortic insufficiency and rheumatoid spondylitis. In: Progress in Arthritis, pp. 154 to 176. Edited by Talbot, J. R. New York, 1958. Grune & Stratton, Inc.)

FIG. 6. Case 1. Focal linear loss of elastic tissue with replacement fibrosis in the media of the aorta. Verhoeff-van Gieson stain.

FIG. 7. Case 1. Vasa vasorum in adventitia showing endarteritis obliterans and chronic perivascular inflammation rich in lymphocytes and some plasma cells.

FIG. 8. Case 1. Sacroiliac joint showing bone destruction and replacement of the articular cartilage by fibrous tissue.

arch. The adventitia showed massive thickening, and the intima contained scattered pale non-calcified plaques. There was an extensive fibrinous adhesive pericarditis. The heart weighed 650 gm., and the left ventricle was thickened. The aortic valve was dilated, measuring 100 mm., and the cusps were slightly thickened at the base, but there was no fusion or widening of the commissures. The trabeculae carneae of the left ventricle were flattened. There were fibrous pleural adhesions with a right hydrothorax of 150 cc.

Microscopically, there was a prominent subendothelial zone of fibrin on the intima, and there were areas of acute and chronic inflammation in the media with infiltration of polymorphonuclear leukocytes around zones of necrosis. (Fig. 5.) Moderate metachromasia of the media was demonstrated with toluidine blue and small areas of destruction of elastic tissue by Verhoeff-van Gieson's stain. Lymphocyte and plasma cell infiltrations were present in many perivascular areas. (Fig. 6.) Fibrosis and perivascular lymphocytic infiltration was present in the adventitia, and there was marked endarteritis obliterans of the vasa vasorum. (Fig. 7.) The necrosis and inflammatory reaction was most prominent in the ascending aorta, but extended to the level of the renal arteries. There was an extensive chronic fibrous pericarditis with foci of "fibrinoid degeneration," and areas of acute inflammation with small areas of perivascular fibrosis in the myocardium.

Examination of the sacroiliac joints revealed replacement of the cartilage by fibrous tissue. (Fig. 8.) Similar changes were seen in the sternoclavicular joints. Synovial tissue from the knee and shoulder joints revealed no abnormalities. Histological study of the nodule from the region of the elbow showed that it consisted of fibroblastic proliferation, histiocytes, lymphocytes and plasma cells. There was a vague appearance suggesting the features of an old rheumatoid nodule, but palisading was not a prominent feature. In the center of the nodule was amorphous acidophilic fibrinoid-like material.

The anatomical diagnoses were as follows: rheumatoid spondylitis; aortic insufficiency; aortitis due to rheumatoid spondylitis; pericarditis; congestive heart failure; peripheral rheumatoid arthritis, possible; Reiter's disease, possible.

CASE II. M. P. M., a fifty year old white man, was admitted to the Medical College of Virginia Hospital in March 1957. For twenty years prior to this admission he had had arthritis which had begun in the right shoulder and grown progressively worse and more extensive. He had been confined to a wheel chair because of the ankylosis of all of the peripheral joints and the spine. There was no history of rheumatic fever, syphilis, iritis or angina pectoris.

The blood pressure at the time of this admission was 90/60 mm. Hg. (During a hospitalization in June 1950 the blood pressure was recorded as 140/70 mm. Hg.) The pulse rate was 80, the temperature 100.4°F. The heart was grossly enlarged, and the cardiac apex was in the anterior axillary line. There was a loud, blowing diastolic murmur which was heard over the aortic valve and along the left sternal border, and an apical systolic murmur. Every joint in his body was ankylosed.

X-ray of the chest showed a greatly enlarged heart with some congestion in the hilar regions. There was calcification of the lateral ligaments of the dorsal spine. (Fig. 9.) An electrocardiogram taken on a previous admission showed a first degree heart block and many nodal extrasystoles. X-ray films of several peripheral joints taken on previous admissions showed changes of advanced rheumatoid arthritis. The STS was negative. About five hours following admission he became cyanotic and died suddenly.

On pathological examination the aorta was slightly dilated and contained several small fibroatherosclerotic plaques throughout. The heart weighed 550 gm., and the left ventricle was thickened. There was slight flattening of the trabeculae carneae of the left ventricle. The aortic valve was dilated, measuring 115 mm., and the free margin of the aortic cusps was slightly thickened and rounded, without fusion. The endocardium of the outflow tract of the left ventricle showed four small semilunar areas of thickening, arranged in the form of a pseudovalve, the concavity of which was directed against the outflow.

Histologically, there was an inflammatory infiltrate of mononuclear cells in the intima and media, and circumscribed hyalinized nodular areas scattered throughout the externa of the aorta. (Fig. 10.) There were areas of focal necrosis with replacement by linear scars in the media. (Fig. 11.) The tunica externa showed a chronic perivascular inflammation rich in lymphocytes and plasma cells, perivascular fibrosis and endarteritis obliterans of the vasa vasorum. (Fig. 12.) Examination of the sacroiliac joints revealed destruction of the articular cartilage and fibrous union of the bones of the sacroiliac joint.

Anatomical diagnoses were as follows: rheumatoid spondylitis; peripheral rheumatoid arthritis; aortic regurgitation; aortitis due to rheumatoid spondylitis; and pseudovalve formation, left ventricle.

CLINICAL DISCUSSION (TABLE I)

Since the most common age at which rheumatoid spondylitis develops is between twenty and twenty-five years, the ages represented in this group would suggest that aortic insufficiency does not usually appear until the disease has been present for several years. Even in Case VII, in which aortic regurgitation was first found when the patient was thirty-two years of age, the history indicated that the spondylitis began some eight years previously. A possible exception

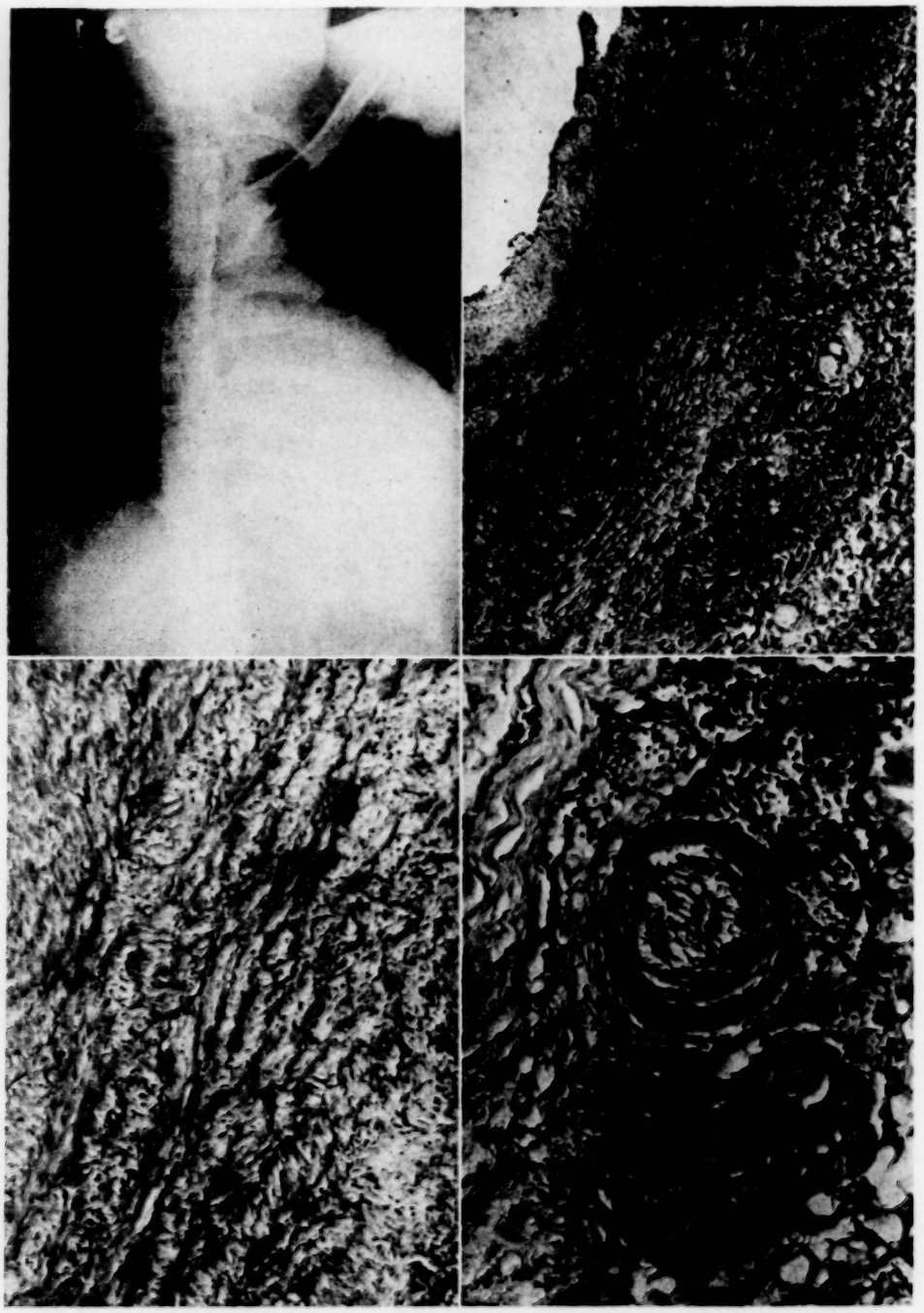

Fig. 9. Case II. Roentgenogram of the chest. The heart is enlarged, and there is calcification of the spinal ligaments. Postmortem examination showed changes of rheumatoid spondylitis in the sacroiliac joints.

Fig. 10. Case II. Low power showing thickening of the intima, loss of elastic tissue in the media, endarteritis obliterans of the tunica externa, and nodular, well circumscribed, well hyalinized, almost sclerosed areas in the tunica externa.

Fig. 11. Case II. Higher magnification of the media showing loss of elastica Verhoeff-van Gieson stain.

Fig. 12. Tunica externa, higher magnification. Endarteritis obliterans of the vasa vasorum, perivascular infiltration rich in lymphocytes and plasma cells, and perivascular fibrosis.

TABLE I
CLINICAL DATA

Case No., Sex and Age (yr.)	Peripheral Rheumatoid Arthritis	Erythrocyte Sedimentation Rate (mm.)	History of Rheumatic Fever	STS*	Blood Pressure (mm. Hg)	X-ray of Heart	Electrocardiogram
I, M, 54	Yes	51	No	Negative	140/50	LVH†	A-V block, first degree LBBB,‡ LVS§
II, M, 50	Yes	..	No	Negative	140/70	LVH	A-V block, first degree
III, M, 51	No	..	No	Negative	160/80	LVH	LVS
IV, M, 51	Yes	24	No	Negative	175/75	LVH	A-V block, first degree Wenckebach
V, M, 48	Yes	30	No	Negative	120/50	LVH	A-V block, first degree
VI, M, 62	No	10	Possible	Negative	160/75	LVH	LVS
VII, M, 32	No	26	No	Negative	182/80	Normal	Normal
VIII, M, 52	Yes	30	No	Negative	140/40	LVH	LBBB, LVS

* Serological test for syphilis.
† Left ventricular hypertrophy.
‡ Left bundle branch block.
§ Left ventricular strain.

to this is Case I; the history of joint disease extended over a period of only three years, and the changes in the sacroiliac joints and lumbar spine seen in the x-ray films would indicate relatively recent development of the spondylitis. (Fig. 4.) Our records were not sufficiently detailed to give us exact information as to the time of onset of the rheumatoid spondylitis and aortic insufficiency in most of the cases, and we have omitted this from our data.

Peripheral rheumatoid arthritis was present in five patients, which is a somewhat higher proportion than reported by Schilder [*18*], and less than reported by Clark [*19*]. The limited number of patients observed in this series would make this incidence of no statistical value, however. The erythrocyte sedimentation rate (Cutler) was elevated in five of the eight patients, was normal in one, and not recorded in two. Iritis was present in one patient (Case IV), and questionably present in another (Case I); in this latter case repeated references were made to the presence of conjunctivitis but nowhere in the record was the term iritis applied to the lesion. A history of urethritis was noted in two patients (Cases I and IV). Small subcutaneous nodules were present in the region of both elbows in one patient (Case I), and clinically appeared to be typical of the subcutaneous rheumatoid nodule. Histological examination, however, did not satisfactorily substantiate this clinical impression.

The STS reaction was negative in each patient, and none had clinical manifestations of syphilis. A positive history of rheumatic fever was not obtained in a single instance. In Case VI rheumatic fever was a possible diagnosis. In this instance the joint disease began in 1918 with migratory polyarthritis, but established itself shortly as a chronic disability of the back. Shortly after this episode a diagnosis of "an enlarged heart" was made, but there had been no previous diagnosis of valvular heart disease. In four patients (Cases I, II, III and VIII) an apical systolic murmur that might be interpreted as mitral insufficiency was present. In two of the patients (Cases I and II) a postmortem examination was obtained and no evidence of mitral valvular disease was noted.

The pulse pressure was in excess of 50 mm. Hg in each case, and in two (Cases VII and VIII) there were peripheral signs of aortic insufficiency (capillary pulsation in the nail bed and Duroziez' sign). X-ray examination of the heart showed evidence of left ventricular hypertrophy in every patient except one (Case VII). One patient (Case I) had evidence of congestive heart failure six months prior to death, and death occurred as a result of this condition. A second patient (Case VIII) had been treated on several occasions for congestive heart failure over a period of two years. One patient (Case IV) was apprehensive about his heart because of symptoms consisting of palpitation, an irregular pulse, spells of dizziness, and moderate shortness of breath. Substernal chest pain was noted in two patients (Cases I and VIII), and each had been

treated with nitroglycerin, one with and one without relief. Pericarditis was noted clinically in one patient (Case I), and confirmed on pathological examination. Five patients presented electrocardiographic evidence of conduction defects either in the form of a first degree A-V block (Cases I, II, IV and V), or a left bundle branch block (Cases I and VIII). There was a Wenckebach's phenomenon in one patient (Case IV). Four patients (Cases I, III, VI and VIII) showed, in addition, an electrocardiographic pattern of left ventricular strain. In only one (Case VII) was the electrocardiographic pattern considered to be within normal limits.

PATHOLOGICAL DISCUSSION

Postmortem examinations were performed in two patients. In both the hearts were dilated, and there was hypertrophy of the left ventricle and flattening of the trabeculae corneae. The coronary orifices were patent. The aortic rings were dilated and incompetent, measuring 100 and 115 mm., the aortic cusps were thickened and the margins slightly rounded, but there was no fusion.

The histological changes present in both patients were more characteristic of those seen in syphilis than in rheumatic fever. The tunica externa showed endarteritis obliterans of the vasa vasorum and perivascular infiltration composed principally of lymphocytes and plasma cells. There was focal destruction of the elastica of the media with replacement by linear scars. The intima was thickened, with focal inflammatory areas composed of polymorphonuclear cells, lymphocytes and plasma cells. The sacroiliac joints in both patients showed destruction of the articular cartilage and replacement with fibrous tissue.

SUMMARY

Aortitis and aortic insufficiency were found in eight patients during the course of examinations made in 265 patients with rheumatoid spondylitis. There was no evidence of syphilis in any of the eight patients, a questionable history of rheumatic fever in one, and evidence of peripheral rheumatoid arthritis in five.

Rheumatoid spondylitis was diagnosed on the basis of typical roentgen changes in the sacroiliac joints, and aortic insufficiency by a diastolic murmur heard over the aortic valve area and along the left sternal border by at least two observers.

In addition to the diastolic murmur of aortic insufficiency and the wide pulse pressure, the following cardiac manifestations were noted: left ventricular enlargement, first degree A-V block, left bundle branch block, Wenckebach's phenomenon, substernal pain suggestive of coronary insufficiency, pericarditis and congestive heart failure. Pathological examination showed the following types of lesions in the two cases: (1) dilated incompetent aortic valves; (2) shortened, thickened aortic valve cusps with rounded margins but with no evidence of fusion; (3) a dilated aorta with the intima wrinkled, thickened, and covered with pale non-calcified plaques; (4) patchy destruction of the elastica of the media with replacement by fibrous tissue; (5) endarteritis obliterans of the vasa vasorum of the tunica externa associated with perivascular infiltration and fibrosis; and (6) absence of any significant damage to the other valves of the heart. These changes were most prominent at the base of the aortic valve cusps and the ascending portion of the aorta. In one case the gross changes extended to the level of the renal arteries.

The findings in these two cases and a review of those previously reported indicate a specific relationship between rheumatoid spondylitis and this type of aortitis.

Acknowledgment: The authors wish to thank Dr. Marta Camilo, Instructor in Pathology, Medical College of Virginia, for the pathological studies in Case II.

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Review

Blood Glucose and the Liver*

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THIS brief review deals with one small segment of mammalian carbohydrate metabolism, namely, the role of the liver in controlling the concentration of circulating glucose. Completeness will be sacrificed in the interest of simplicity, and an effort will be made to interpret the qualities previously assigned to the liver for its ability to regulate blood glucose levels in terms of a few enzyme reactions and their quite logical behavior.

HISTORICAL BACKGROUND

Between 1850 and 1853 Claude Bernard [*19*] described glycogen as a temporary storage form for carbohydrate and discovered the ability of the liver to maintain blood glucose levels in the absence of carbohydrate in the diet. Further major contributions had to await the development of adequate methods for the determination of glucose in biological fluids. Soskin and his coworkers [*139*] refined the observations of Bernard and demonstrated that the perfused dog liver removed glucose from the blood stream when portal blood contained high glucose levels. Conversely, when the portal blood was low in glucose, glucose was produced by the liver. (Fig. 1.) The rate of removal or production was found to be directly proportional to the degree of hyper- or hypoglycemia [*71*]. Bondy, James and Farrar [*23*], using the technic of hepatic vein catheterization, found that the splanchnic bed in man reacted similarly by removing glucose when hyperglycemia was present.

Since hepatic vein catheterization studies represent the summation of hepatic, intestinal and mesenteric metabolism, a more direct approach has been devised whereby the amount of glucose entering and leaving the blood can be calculated by assaying the concentration and specific activity of blood glucose after injection of a tracer dose of radioactively-labeled glucose. With this technic, Searle and Chaikoff [*133*] found that hyperglycemia inhibited hepatic glucose production. Another, perhaps more direct technic was previously used by Cherry and Crandall [*32*] who sampled blood through London cannulas and found that the liver removed glucose from the hyperglycemic dog.

The experimental data defining the liver as the principal source of glucose production is voluminous and well known, and need not be cited here. The liver, therefore, can either produce or remove glucose. Only the technics (not the facts) have advanced since Claude Bernard made his original observations and interpretations. How does the liver assay and respond to the concentration of circulating glucose? Is this a function of the cell membrane or is this a function of the intracellular enzyme systems? And what influences do hormones exert upon this already complex balance? These are questions to which answers have been sought with modern methodology and which will be discussed in this review.

GLUCOSE PENETRATION

Peripheral Tissues. Recently Levine et al. [*90,91*], Ross [*126–128*] and Drury and Wick [*42*] have described the rate-limiting role of the cellular membrane in carbohydrate metabolism in peripheral tissues. Except under unusual experimental conditions, freshly excised muscle from normal or diabetic animals contains only that amount of free glucose which is expected from its

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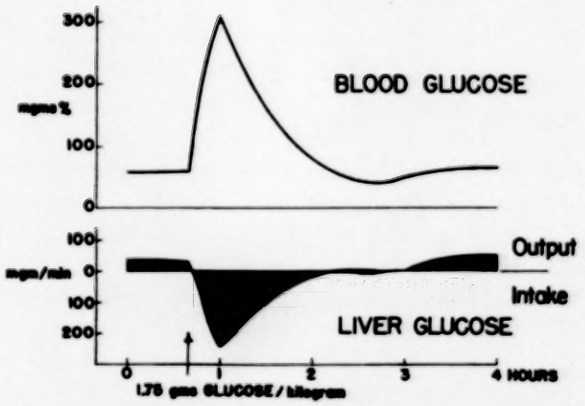

FIG. 1. An experiment from Soskin et al. [*139*] showing the relationship between the level of blood glucose and the output and intake of glucose by the liver before and after the injection of 1.75 gm. glucose/kg.

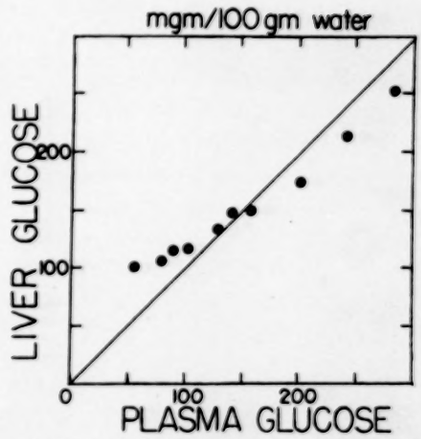

FIG. 2. Relationship of glucose concentrations in rat plasma and liver after injection of varying amounts of glucose. The intercept where both concentrations are equal approximates 150 mg./100 gm. water [*30*].

volume of extracellular fluid [*45,89,151*]. Park et al. [*111,112*] have demonstrated small amounts of free intracellular glucose in muscle during extreme hyperglycemia or hypothermia. They also reported that this amount of free intracellular glucose is increased by the administration of insulin. Numerous other papers have appeared demonstrating the existence of a carrier system which is insulin-sensitive, and only relatively specific for glucose, and which appears to be catalytic but not energy requiring [*48,76, 101,102,124*]. Previous studies [*145*] have demonstrated insulin-binding to the cell membrane, and Zierler [*169*] has recently suggested that the primary effect of insulin may directly alter the membrane potential. There is also evidence for a less specific mode of carbohydrate entry into muscle which is not insulin-sensitive and approximates some of the characteristics of diffusion. For a more extensive discussion of this complicated picture, the reader is referred to the recent review by Ross [*128*].

Liver. If the cell membrane were to play an important role in hepatic glucose metabolism, it should either offer resistance to diffusion, as in the case of peripheral tissues, or provide active transport of glucose in at least one direction. On the other hand, a membrane allowing free equilibration between glucose inside the cell and glucose in the circulating fluid would enable intracellular enzyme systems to determine the rate and direction of glucose metabolism in the liver.

Following the development of adequate analytical methods, the visceral organs were found to contain high quantities of free glucose when compared to carcass [*50*]. Recently, Gey [*54*] reported that liver contained the highest quantity of free glucose of any of several tissues analyzed in the rat, concentrations approximately equal to the concentration of glucose in the blood.

Using the difference in reducing substance before and after treatment of a deproteinized sample of tissue with purified glucose oxidase, it has recently been found that in the presence of plasma levels of about 160 mg./100 gm. H_2O (i.e., 150 mg. per cent) the concentration of free glucose in liver tissue from normal animals is equal to that in plasma [*30*]. Above this plasma concentration, glucose concentration in liver is less than that in plasma; below this concentration that of plasma is less than that in liver. This relationship, expressed as mg. per 100 gm. H_2O, is demonstrated in Figure 2. It is apparent that these results are in excellent agreement with the experiments of Soskin et al. [*139*] in which no net difference between the rate of removal and rate of production of glucose by the liver was observed at blood glucose concentrations of approximately 150 mg. per cent.

Further experiments have shown that other hexoses such as fructose, mannose and galactose [*30*] and pentoses [*30,66,99,131*] freely enter the liver cell either *in vivo* or *in vitro*. Other substances which lack ketone or aldehyde groups, such as mannitol, sorbitol or glycerol, also freely penetrate the liver cell. Di- and trisaccharides are restricted to the calculated extracellular space, whether or not they exhibit reducing properties. (Fig. 3.) It would appear that the penetration of

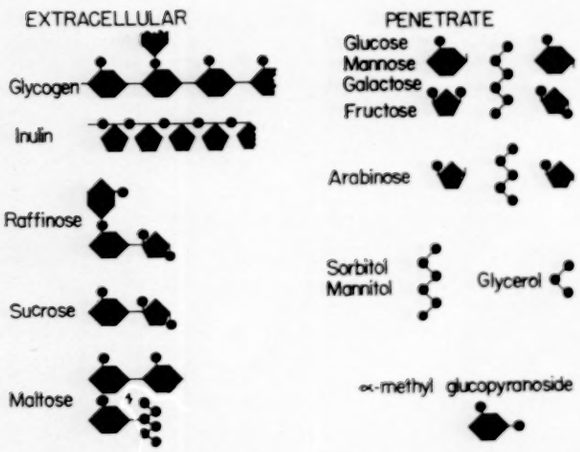

FIG. 3. Figurative silhouettes of various substances tested for *in vivo* penetration into liver. The poly-, tri- and disaccharides listed on the left are restricted to extracellular fluid. Maltose is presented as the double pyranose form and as the open-chain reducing form. The molecules diagrammed on the right penetrate freely. Glucose, mannose, galactose and fructose are drawn as open chain compounds and also as both alpha and beta furanose (pentagonal) and pyranose (hexagonal) forms. Arabinose is shown as the open chain and both furanose forms. Glycerol, sorbitol and mannitol, being polyalcohols, exist only as open chains. The last molecule, α-methyl-glucopyranoside, freely penetrates the liver cell but is a rigid closed-ring structure.

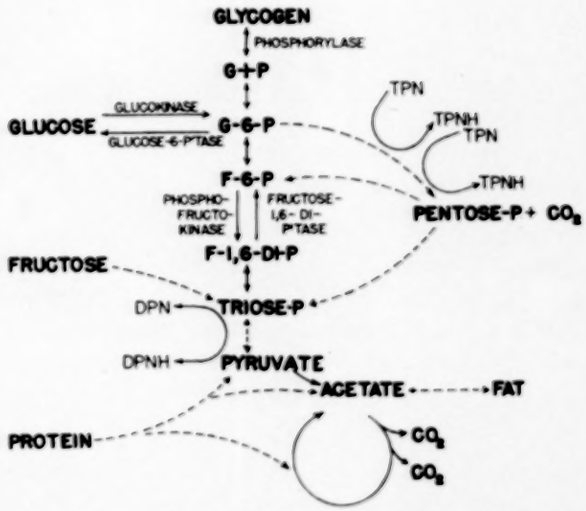

FIG. 4. Abbreviated chart of hepatic carbohydrate metabolism defining the reactions which are considered in this paper. Multiple enzyme systems are drawn as broken lines. The tricarboxylic acid (Krebs) cycle is sketched as a circle. (G-1-P = glucose-1-phosphate, G-6-P = glucose-6-phosphate, F-6-P = fructose-6-phosphate, F-1, 6-P = fructose-1,6-diphosphate, triose-P = triose phosphate.)

carbohydrate into liver, unlike that into muscle, is primarily dependent on molecular size.

Other experiments have shown rapid equilibration of radioactive glucose between liver water and extracellular water, whether the net flow of glucose was into the liver cell as in hyperglycemia, or out of the liver cell as in hypoglycemia, again suggesting free diffusion as the method of glucose flow across the cell membrane. This free exchange was also found in the alloxan-diabetic animal [*30*].

PRIMARY REACTIONS

Since glucose penetration into or out of the liver cell is not a rate-limiting step, the enzyme systems which control the entry and exit of glucose into and from the pool of metabolic intermediates assume prime importance. The nature, function and location of these intracellular enzymes will be briefly reviewed. Their relationships are summarized in Figure 4.

Glucokinase. To enter the metabolic pool, glucose must first be enzymatically phosphorylated to form glucose-6-phosphate. This enzyme in liver will be referred to as "glucokinase" rather than "hexokinase," since the liver also contains specific kinases for other hexoses such as fructose [*70,113*] or galactose [*80*] as well as for other carbohydrates such as ribose [*1*] and also glycerol. Partial purification of a liver enzyme catalyzing the phosphorylation of glucose has been described [*39*], but further characterization has been difficult due to its apparent lability and difficulties in assaying its activity. For this reason presently available values for the rate of glucose phosphorylation in hepatic tissue have been derived from calculations based on results obtained during incubation of tissue slices [*120*] rather than from direct assays of enzymatic activity in liver homogenates.

That glucokinase is a specific entity is indicated by the specific reduction of calculated glucose phosphorylation in the diabetic state [*120*]. In contrast, the utilization of fructose [*7,120*], pyruvate [*119*] and glycerol [*7*] by hepatic tissue slices from diabetic animals is unaltered, and it has been inferred that the specific kinases for these substrates are unchanged in the insulin-deficient animal. Similarly, fructose and galactose tolerance curves are similar in diabetic and normal animals, e.g., rabbits [*16*] and man [*98*].

It is assumed that hepatic glucokinase catalyzes the reaction:

$$\text{Glucose} + \text{ATP} \rightleftharpoons \text{Glucose-6-phosphate} + \text{ADP}$$

The reaction utilizes adenosine triphosphate (ATP) and generates adenosine diphosphate (ADP). The free energy change involved (ΔF) makes the reaction, under physiological conditions, essentially unidirectional, in favor of glucose-6-phosphate formation [*125*]. The enzyme is found in the soluble cytoplasm after sedimentation of particulate structures such as nuclei, mitochondria and microsomes [*39*]. The metabolic variations of this enzyme and its insulin-dependence will be discussed later.

Glucose-6-phosphatase. The liver is not only unique in possessing an insulin-sensitive phosphorylation mechanism for glucose, but also differs from most other tissues by having a specific enzyme capable of catalyzing the hydrolysis of glucose-6-phosphate once it is formed [*35,46*]. Although this enzyme, glucose-6-phosphatase, is also found in renal proximal tubules and to a limited degree in other tissues [*33,41,157*], its major physiological role would appear to result from its presence in liver where it provides for the controlled release of glucose from glucose-6-phosphate. Glucose-6-phosphatase catalyzes the following reaction:*

$$\text{Glucose-6-phosphate} + H_2O \rightleftharpoons \text{Glucose} + \text{Phosphate}$$

Like glucokinase, glucose-6-phosphatase catalyzes an essentially unidirectional reaction under physiological conditions, with the equilibrium in favor of formation of free glucose and inorganic phosphate. Unlike glucokinase, it is almost totally sedimented after ultracentrifugation, and is found in the microsomal fraction [*41,68,69*] which in the intact cell is the reticular substance branching and folding throughout the cytoplasm [*110*]. Recent studies have shown that the activity of this enzyme varies according to the metabolic state of the animal, as will be discussed in a later section.

The Steady State. The liver therefore provides separate catalysts for two opposing reactions, each having a physiological effect which is operationally unidirectional. Both are located inside a cell freely permeable to glucose. If intracellular glucose is freely accessible to glucokinase, and glucose-6-phosphate to glucose-6-phosphatase, the rate and direction of metabolic flow should be influenced by four major factors: (1) the activity of glucokinase, (2) the activity of glucose-6-phosphatase, (3) the concentration of free glucose and (4) the concentration of glucose-6-phosphate inside the liver cell.* It would appear from available data that this is the case.

In the normal animal with a blood glucose concentration of 150 mg. per cent, a steady state is achieved whereby the reaction resulting in glucose uptake and that in glucose release are occurring at equal rates. A change in the activity of either enzyme or a change in the concentration of either substrate alters this steady state.

It should be stressed that even without any change in the activity of either enzyme the liver is able to maintain a steady level of blood glucose since hyperglycemia presumably causes a rise in intracellular glucose-6-phosphate concentration and a resultant increase in glycogen synthesis. Conversely, hypoglycemia presumably decreases the concentration of glucose-6-phosphate, with a resultant mobilization of glycogen. The mobilization of glycogen may take place in the adrenalectomized animal and therefore is not necessarily dependent upon a response to epinephrine [*154*].

It might be thought that a single enzyme would have sufficed for the regulation of the concentration of circulating glucose but nature has provided a more versatile arrangement with two "unidirectional" enzymatic reactions. A single enzyme can only accelerate the attainment of a given equilibrium, and an increase or decrease in enzyme activity only changes the rate of the reaction. On the other hand, two separate enzymatic reactions controlling a given step, but in opposing directions, can change not only the rate but also the direction of net

* A second reaction (personal communication from L. F. Hass and W. L. Byrne) believed to be mediated by glucose-6-phosphatase is:

$$\text{Enz} + \text{G-6-P} \rightleftharpoons \text{Enz-G-6-P} \rightleftharpoons \text{Enz-P} + \text{G}$$

Such a sequence of reactions would be consistent with the observed exchange of C^{14} between labeled glucose and unlabeled glucose-6-phosphate by liver microsome preparations, as well as the competitive inhibition of hepatic glucose-6-phosphatase activity by glucose. It would therefore appear possible that high levels of glucose could directly reduce hepatic glucose output by inhibiting the hydrolysis of glucose-6-phosphate catalyzed by glucose-6-phosphatase.

* Other limiting factors in the glucokinase reaction might result from the availability of ATP or the presence of the proper ionic environment. However, only in the most severe metabolic derangements, such as prolonged anoxia or high concentrations of dinitrophenol, do these factors appear to become rate-limiting.

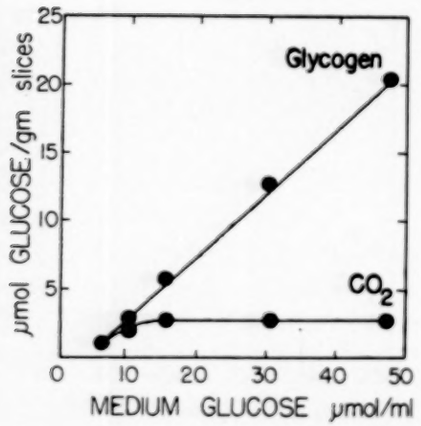

FIG. 5. Glucose uptake by liver slices parallels the concentration of glucose in the medium. Glycogen synthesis is proportionate to uptake but the pathway of glucose oxidation to carbon dioxide becomes saturated at low levels of glucose uptake [*29*].

flow as well as the final ratio of the two substrates when a steady state is achieved. When one (or both) of these enzymes is under hormonal control (either directly or indirectly) that pathway of metabolism loses its autonomy and is subject to distant control. It therefore can be integrated into other more remote metabolic patterns of the whole organism. Such is the case for these two enzymes of liver tissue.*

GLYCOLYSIS

Hyperglycemia and hypoglycemia have, so far, been shown to result in glycogen deposition or glycogen mobilization, respectively, by expanding or diminishing the effective concentration of glucose-6-phosphate. If liver were an actively glycolyzing tissue this conservation of hexose would not be possible in the face of a constant catabolic drain, with formation of lactate or carbon dioxide. Rapid glycogen mobilization by glucagon or rapid glycogen deposition in the severely hyperglycemic animal does not increase the concentration of lactate in hepatic vein blood, suggesting a rate-limiting step in glycolytic pathways [*27,32*]. Also, whereas the uptake of glucose from the medium by liver slices and its incorporation into liver glycogen is proportional to the glucose concentration of the medium, the pathways of glucose catabolism to lactate or carbon dioxide soon become saturated [*29*]. (Fig. 5.) Since fructose, which enters the glycolytic sequence as triose phosphate (Fig. 4) is converted to carbon dioxide at a rate exceeding the maximal rate for glucose both in slices [*120,167*] and in the perfused liver [*83*], the presence of a rate-limiting step between glucose-6-phosphate and triose-phosphate is indicated.

Olson [*109*] recently reviewed the data on hepatic glycolysis and concluded that the rate-limiting step was the interconversion of fructose-6-phosphate and fructose-1,6-diphosphate, since the addition of fructose-1,6-diphosphate led to increased glycolysis. Previous experiments by LePage [*88*] demonstrated that fructose-1,6-diphosphate caused maximal rates of lactate production in tissue homogenates, including liver. Olson assayed phosphofructokinase, which converts fructose-6-phosphate to fructose-1,6-diphosphate (Fig. 4) in liver and other tissues and found the activity of this enzyme to be lowest in liver, a fact in keeping with the poor glycolytic activity of this tissue. Recent studies using reconstituted biological systems have also suggested that this step may control the over-all rate of glycolysis [*2*].

The reconversion of fructose-1,6-diphosphate to fructose-6-phosphate requires a second enzyme, fructose-1,6-diphosphatase [*58*]. Thus, as in the case of glucose and glucose-6-phosphate interconversion, two "unidirectional" reactions control the metabolic flow, and again, if one or both of these enzymes can be altered by hormonal influences, the net rate and direction of the glycolytic sequence is subject to distant metabolic control. Recently, Mokrasch, Davidson and McGilvery [*100*] reported that fructose-1,6-diphosphatase activity is indeed increased by fasting, protein or fructose feeding, or corticosteroid administration.

Thus the physiological observation that liver exerts poor glycolytic activity may be the result of its enzymatic architecture, since a rate-limiting step controls the formation of fructose-1,6-diphosphate from fructose-6-phosphate. Whether the adult liver under aerobic conditions ever exhibits a net carbohydrate flow in the direction of glucose catabolism is questionable, since even resting muscle continuously produces lactate [*5,168*] which must be cleared by the liver and reconverted to hexose. Conversely, as is

* From thermodynamic considerations, Krebs [*82*] has pointed out the practical necessity of having two different enzymes to achieve glucose phosphorylation and dephosphorylation. This is also true for the interconversion of fructose-6-phosphate and fructose-1,6-diphosphate, and of phosphoenol-pyruvate and pyruvate. If glucose synthesis from pyruvate were to result from reversal of the kinase reactions with generation of ATP from ADP, excessively high concentrations of pyruvate, far exceeding physiological concentrations, would be required.

well known, glucogenesis can proceed at extremely rapid rates [*14,162*]. Whether glycolysis or glucogenesis (and the respective rate of each) is favored would depend in each instance on the relative excess or deficit of fructose-1,6-diphosphatase activity when compared to the activity of phosphofructokinase.

Oxidative pathway: Quantitatively speaking, the contribution to the metabolism of glucose-6-phosphate by the direct oxidative pathway (also termed the "shunt," the "pentose phosphate pathway," the "phosphogluconate oxidation pathway," etc.) is less in liver than in glycogen synthesis, glycolysis or glucose production. Recent experiments [*104*] in the perfused rat liver have demonstrated that the oxidation of added glucose-C^{14} accounted for only 2.5 per cent of total hepatic carbon dioxide production. These authors [*103*] also found that *in vivo* the direct oxidative pathways accounted for about one-third of the lactate formed from glucose. From experiments in liver slices, *in vitro*, it can be calculated that the contribution of an extra-glycolytic pathway to the formation of lactic acid and fatty acids is slight. Approximately 86 per cent of lactic acid and fatty acid formed from labeled glucose by liver slices is derived via glycolysis [*9,12,22*].

It can be calculated that of every sixteen molecules of glucose phosphorylated by hepatic glucokinase, eight are dephosphorylated by glucose-6-phosphatase action, four are incorporated into liver glycogen, three are glycolyzed, and of these, one may be oxidized by the tricarboxylic acid cycle. The remaining one phosphorylated glucose molecule is oxidized by the direct oxidative pathway. It would appear, therefore, that the direct oxidative pathway may account for a significant fraction of glucose oxidized, although it represents but a minor metabolic fate of the total available glucose-6-phosphate pool, when compared with other available routes of metabolism. (For an extensive and critical review and evaluation of the direct oxidative pathway, see Wood [*166*].) The quantity of glucose which is oxidized by this pathway is limited by the availability of oxidized triphosphopyridine nucleotide (TPN) [*29,163*]. This coenzyme (Fig. 4) is required for the first two steps in the oxidation and removal of the aldehyde carbon of glucose as carbon dioxide.*

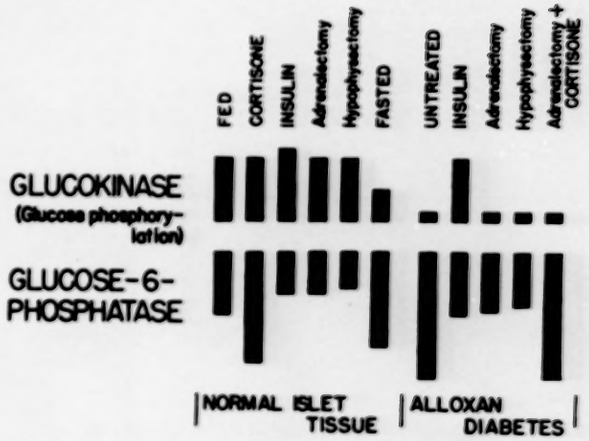

FIG. 6. Comparative values for glucokinase (glucose phosphorylation) and glucose-6-phosphatase in livers from rats. All hormone alterations were in the chronic state. Values derived from references in the text.

GLUCOKINASE AND INSULIN

Using the distribution of radioactively-labeled fructose into both glycogen and glucose of the medium as an index of glucose-6-phosphate metabolism, Renold, Hastings and Nesbett [*120*] have estimated glucokinase activity by calculating the amount of radioactive glucose phosphorylated in paired liver slice incubations. Figure 6 includes data from several papers concerning relative rates of glucose phosphorylation in various metabolic states [*12,14,85,118,120, 121,141–143*]. It is apparent that the intensity of this reaction is dependent on the presence of insulin and is not affected by the presence or absence of adrenal or pituitary glands.

The relation in time of hepatic glucokinase

* Whereas the conversion of fructose-6-phosphate to fructose-1,6-diphosphate appears to be rate-limiting in liver, much evidence suggests that the rate-limiting reaction of glycolysis in other tissues is the dehydrogenation of phosphoglyceraldehyde, a reaction requiring diphosphopyridine nucleotide (DPN) as coenzyme. It has been reported that the major fraction of DPN in liver is in the oxidized form [*56*], suggesting that its oxidation is not a rate-limiting phenomenon, whereas up to 97 per cent of TPN in liver is present in the reduced form, again suggesting that its reoxidation may be the rate-limiting step of the direct oxidative pathway. Both of these limiting steps can theoretically be bypassed by transketolase and transaldolase reactions with fructose-6-phosphate as one of the substrates, thereby forming intermediates of the direct oxidative pathway below the level of the initial oxidative steps. As yet there are no data from which a quantitative assessment of this metabolic route can be calculated, although recent experiments measuring the incorporation of specifically-labeled glucose into DPN-ribose suggest that only 5 to 10 per cent of ribose synthesis occurs via the direct oxidative steps and the remainder is derived from fructose-6-phosphate via transketolase and transaldolase reactions [*137*]. (For further definition of these steps, see Horecker and Hiatt [*72*]).

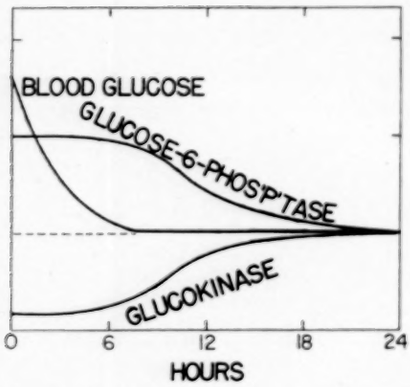

FIG. 7. Time-sequence of changes in hepatic glucose-6-phosphatase, glucose phosphorylation (glucokinase activity) and blood glucose after administration of insulin to diabetic rats [*8,121*].

activity in the presence of insulin differs greatly from the effect of this hormone on glucose assimilation in muscle. Exposure of muscle to insulin *in vivo* or *in vitro* results in immediate acceleration of glucose metabolism [*81,146*] But insulin given *in vivo* requires approximately six to twelve hours before a definite increase in hepatic glucose phosphorylation can be demonstrated [*121*]. Abrupt withdrawal of insulin likewise results in a gradual fall in hepatic glucose phosphorylation during the following forty-eight to ninety-six hours [*142*].

An immediate insulin effect in decreasing hepatic glucose production has recently been reported [*44,117, 155*]. This conclusion was derived from the demonstration of an early decrease in the rate of endogenous dilution of infused radioactive glucose following the administration of insulin to dogs. However, it would also seem possible that the outer tiers of hepatic glycogen acquired radioactivity by equilibration with the infused radioactive glucose [*148,149*] and rapid glycogenolysis resulting from insulin-induced hypoglycemia returned this labeled glucose to the blood stream. The observed slowing of the fall in blood glucose specific activity would then be interpreted as due to decreased glucose production as also observed and discussed by Wall and his associates [*155*]. In addition, a return of labeled glycolytic products (i.e., lactate) from the periphery and the incorporation of this labeled carbon into glucose might have occurred. Recent experiments in our laboratory using glucose labeled with C^{14} in carbon 6 of the glucose molecule have indicated a considerable recycling of C^{14} through three carbon fragments and resynthesis of glucose from these fragments. The administration of insulin greatly accelerates this process.

Henderson et al. [*67*] found a gradual and delayed decrease in glucose entry into the blood stream in the insulin-treated depancreatized dog. Hepatic glycogen storage is much less in this preparation and the error resulting from glycogen labeling would therefore be diminished. This gradual change is in agreement with the slow increase in glycogen phosphorylation noted in the insulin-treated alloxan-diabetic rat [*121*]. (Fig. 7.)

Liver perfusion studies have shown either no acute effect of insulin [*95*], a paradoxical increase in glucose production [*114*], or a slight glucose uptake after one hour of perfusion with insulin in the perfusing medium [*59,60*]. Studies using arterial hepatic venous differences measure not only hepatic metabolism but also the metabolism of highly insulin-sensitive mesenteric adipose tissue in addition to other visceral organs. Recent studies using dogs which were maintained for weeks with catheters placed in the portal vein, the hepatic vein and the splenic artery have failed to reveal an acute effect of insulin upon glucose uptake in liver. Furthermore, whereas decreased glucose production was not observed, rapid glycogenolysis and increased glucose production were invariably seen, whether insulin was injected peripherally or intraportally [*11,96*]. On the other hand, Madison and Unger [*170*] recently reported that insulin injected intraportally or into a peripheral vein produced the same degree of hypoglycemia; however, there was a greater peripheral arteriovenous difference following the peripheral administration of the insulin. They stated that this suggested a greater hepatic effect following portal administration, thereby inferring a direct effect of insulin on decreasing net hepatic glucose output.

Berthet et al. [*21*] have reported an increase in glucose incorporation into glycogen after addition of insulin to rabbit liver slices. This observation has been confirmed in our laboratory, although repeated attempts to demonstrate an effect of insulin *in vitro* on glucose metabolism by *rat* liver slices have failed to reveal significant changes in glucose uptake, output, oxidation or incorporation into glycogen [*121*]. (For a detailed review of insulin and hepatic metabolism, see Levine and Fritz [*92*].)

In the intact organism, the sluggish response of hepatic glucokinase activity to insulin may serve as a physiological buffer for abrupt changes in blood glucose levels. For example, if hepatic glucokinase activity paralleled glucose assimilation by muscle and other peripheral tissues, severe hypoglycemia might be expected to follow the hyperinsulinemia produced by a meal rich in carbohydrate.

FASTING

More prolonged episodes of carbohydrate deprivation are associated with a marked decrease in hepatic glucokinase activity, perhaps as a result of a persistently low secretion of insulin. The steady state "balance" of the two enzymatic reactions is altered in favor of hepatic glucose production. Evidently, this same increased glucose production could occur without changes in enzyme activities as a result of hypoglycemia, which would favor glycogen mobilization on the basis of decreasing concentrations of glucose-6-phosphate. The less radical response allows for increased glucose

production without a severe fall in blood glucose, thereby protecting tissues such as brain, which require adequate blood glucose levels at all times.

Fasting is accompanied by changes in the activities of other enzymes as well. Glucose-6-phosphatase increases [*6,8,86,156*], thereby augmenting the effect of decreased glucokinase in facilitating hepatic glucose production. Fructose-1,6-diphosphatase activity also increases [*100*], a change consistent with acceleration of gluconeogenesis from hexose precursors. The over-all effect of these changes is to expedite glycogen mobilization and glucose production and to increase glucogenesis to supplement and replace the limited supply of glycogen stores.

DIABETES

Metabolically speaking, the diabetic liver is a fasting liver in which biochemical alterations have become grossly exaggerated. Although peripheral glucose assimilation in the absence of insulin is markedly decreased, this defect may be largely overcome by increasing blood glucose concentrations, as has been demonstrated and emphasized by the elegant studies of Soskin and Levine [*140*]. Indeed, in all but the most severe states of insulin deficiency a new equilibrium with essentially normal rates of peripheral glucose assimilation—albeit obtained at much higher blood glucose concentrations—is reached [*75,138,140*]. These increased levels of blood glucose are provided at the expense of increased hepatic glucose production.*

In diabetes, hepatic glucokinase activity falls to one-fifth to one-tenth of normal, again presumably as a result of insulin-lack, and glucose-6-phosphatase activity doubles. The steady state at which both enzyme systems turn over the same number of glucose molecules is reached only at higher and higher levels of blood glucose. In the presence of mild diabetes this "steady state" level may be reached at an extracellular fluid concentration of, for example, 250 mg. per cent. Under these conditions postprandial elevations of blood glucose to 350 mg. per cent result in hepatic glycogen deposition, while decreases to 150 or 200 mg. per cent in the postabsorptive state result in glycogen mobilization. The patient with severe diabetes may never reach the required high level of blood glucose concentration at which his liver would cease to produce glucose, since each increase in glucose concentration merely causes a relatively greater increment in urinary glucose loss. Sacks [*129,130*] has measured the concentration of glucose-6-phosphate in normal and alloxan-diabetic rat livers and found the latter to contain about 30 per cent less glucose-6-phosphate, a finding consistent with the slightly decreased glycogen levels seen in this preparation when compared to normal fed animals.

The administration of insulin to the diabetic rat restores glucose phosphorylation to normal and results in a net decrease in glucose-6-phosphatase activity; however, these changes follow by several hours the prompt fall in blood glucose. (Fig. 7.) A similar lag in decreasing gluconeogenesis, as reflected by conversion of radioactive pyruvate to glucose, follows the injection of insulin, normal values for gluconeogenesis being obtained at about the same time that glucose phosphorylation and glucose-6-phosphatase activity have been returned to normal [*8,121*].

Further evidence suggesting that a major and possibly primary impairment of glucokinase activity exists in the diabetic rat has been obtained from studies with two glucose analogues, glucosamine and N-acetylglucosamine. These compounds, which have been shown to inhibit glucose phosphorylation by brain hexokinase [*38*], markedly inhibit glucose metabolism by

* As previously discussed, a means of carbohydrate entry into muscle has been demonstrated which is insulin-insensitive and has some of the characteristics of restricted diffusion. Hyperglycemia may cause enough entry of glucose by this process to compensate for the diminished glucose transport by the more active insulin-sensitive route. Recent studies with radioactively-labeled glucose have been confusing, but in general rates of glucose assimilation and oxidation by the whole animal as measured in milligrams or grams per minute or hour are of the same order of magnitude in the diabetic and in the normal organism [*47,134,135*], in agreement with the initial postulates of Soskin and Levine. Some experiments, however, have shown decreased glucose oxidation in the diabetic state [*147*]. These data may not be conflicting, since in the former experiments tracer doses of labeled glucose were used (thereby allowing the hyperglycemia of the diabetic animals to provide normal oxidation) while in the latter experiments relatively large amounts of glucose were infused, making both normal and diabetic animals hyperglycemic. Indirect evidence that peripheral glucose assimilation is similar in the normal and the diabetic state results from the circumstantial finding that splanchnic glucose production is equal in these two states, as measured by hepatic vein catheterization studied in man [*17,24,105*].

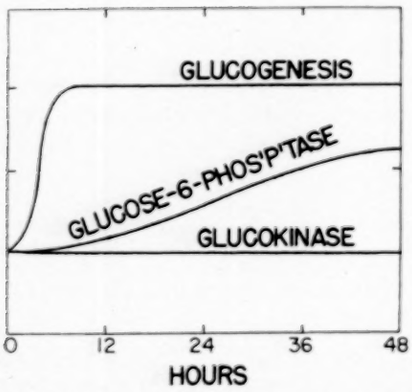

FIG. 8. Time-sequence of events after administration of hydrocortisone to adrenalectomized-diabetic rats. Hepatic glucokinase is unaffected; glucogenesis, as measured by pyruvate incorporation into glucose, rapidly increases and is followed by a rise in glucose-6-phosphatase activity [*8*].

liver slices from normal animals [*144*]. Not only does the presence of glucosamine or N-acetyl-glucosamine result in decreased glucose phosphorylation, but also in decreased glucose oxidation to carbon dioxide, and markedly decreased fatty acid and glycogen synthesis. Indeed, the metabolic pattern of glucose metabolism in hepatic tissue can be converted *in vitro* to that of the diabetic tissue by incubating tissue slices in the presence of these inhibitors. Such observations strongly suggest that the primary lesion in the liver of the diabetic animal is diminished glucokinase activity, and that all other alterations are secondary to this primary lesion.

GLUCOCORTICOIDS

Removal of the adrenals from the diabetic animal improves the diabetic state both of the intact animal and of its subsequently isolated liver [*63,93,119*]. Glucose-6-phosphatase activity returns to normal [*8*] and the discrepancy between its activity and that of glucokinase is decreased. However, this "improvement" results from failure of gluconeogenesis similar to that characteristic of the adrenalectomized animal with intact beta-cells. The liver of the diabetic-adrenalectomized organism exhibits not only decreased glucose uptake, presumably due to the depressed glucokinase activity of insulin deficiency, but also fails to supply adequate amounts of newly formed glucose when required, as during episodes of fasting. Since glucose-6-phosphatase activity, although reduced, is still in relative excess, this animal also fails to store glycogen in adequate amounts, again as in the case of the adrenalectomized organism with intact beta-cells [*34*]. What little glucose-6-phosphate is formed from the meager available supply of precursors is immediately dephosphorylated and carried away by the blood stream. Thus the "amelioration" of the diabetic state produced by superimposed adrenalectomy leaves this animal's liver unable to buffer either hypo- or hyperglycemia. Hypophysectomy superimposed on diabetes produces changes in hepatic carbohydrate metabolism similar to those just described for adrenalectomy [*143*], a reduction in glucose-6-phosphatase activity, again without restoration of the decreased levels of glucose phosphorylation.

The administration of glucocorticoids to normal animals increases gluconeogenesis [*79*] and may produce a sustained rise in blood glucose, a state sometimes called "steroid diabetes" [*73,94*]. This hyperglycemic condition is accompanied by a rise in hepatic glucose-6-phosphatase activity [*8,158,159*], but glucokinase activity remains normal [*14,118*]. Here again, as in the glucocorticoid-treated adrenalectomized animal, the increase in glucose-6-phosphatase activity apparently represents a secondary event since it follows rather than precedes (Fig. 8) increased glucose production [*8,14*]. Animals fed galactose, fructose, high protein or high fat diets also show elevated levels of glucose-6-phosphatase, again suggesting that this enzyme adapts secondarily to states requiring increased glucose production by the liver [*51,52,58*]. The primary site of glucocorticoid action is therefore assumed to be located below the level of glucose-6-phosphate in the sequence of metabolic reactions. Fructose-1,6-diphosphatase activity similarly increases with glucocorticoid therapy [*100*]; data concerning the place of this metabolic event in time have not as yet been reported. However, since it also occurs after fructose or protein feeding, the increase after glucocorticoids is probably also an indirect effect of the hormone, similar to the effect on glucose-6-phosphatase.

Increased peripheral mobilization of amino acids in the presence of the adrenal gland has also been suggested [*25,74,77*], and Bondy, Ingle and Meeks [*26*] reported an accelerated rise in serum amino acid concentration after the administration of glucocorticoids to the eviscerated rat. These studies have been interpreted as possibly indicating a primary action of these

hormones on protein catabolism in peripheral tissues. A recent publication [*108*] has suggested that glucocorticoids directly affect the ability of the liver to concentrate amino acids from the serum.

Attempts to demonstrate an *in vitro* effect of glucocorticoids on isolated liver preparations have met with varying and in general rather insignificant degrees of success [*122*]. However, changes in hepatic metabolism have been observed within two to four hours after administration of glucocorticoids to adrenalectomized diabetic rats [*8*]. As previously mentioned, it is entirely possible that all known alterations of hepatic metabolism occurring after the administration of glucocorticoids are due to adaptive changes of hepatic enzyme activities in response to an increased or altered supply of substrates [*78*]. Experiments by Glenn et al. [*55*] suggesting that the effects of steroids on carbohydrate metabolism are more closely related to the amount of hormone leaving the liver than the amount entering it further support the concept of a primary effect of these hormones on peripheral tissues.

Winternitz, Dintzis and Long [*164*] found a greater loss of carbohydrate in epinephrine-treated adrenalectomized rats than in epinephrine-treated intact control animals. Lactate infusion likewise resulted in less hepatic glycogen deposition in the adrenalectomized animal. These defects were corrected by glucocorticoid replacement. From these data the authors inferred that the lack of glucocorticoids directly affects hepatic metabolism by diverting carbohydrates or their precursors into pathways of metabolism other than glycogen synthesis. These data can also be reconciled, however, with the concept of primary action of glucocorticoids on peripheral tissues followed by secondary adaptation of hepatic enzyme concentration to accelerated gluconeogenesis. Proportionally increased oxidation of lactate by the adrenalectomized animal might then result from lower levels of fructose-1,6-diphosphatase activity leading to a decreased rate of conversion of lactate to glucose and glycogen. Winternitz and Kline [*165*] indirectly demonstrated greater than normal carbohydrate oxidation after epinephrine administration to adrenalectomized animals on the basis of measurements of respiratory quotient changes. (For a recent review of the metabolic effects of the steroids, see Thorn et al. [*150*].)

OTHER FACTORS

In addition to adrenal cortical steroids, insulin-lack and glucose-free diets, thyroid and growth hormones also increase glucose-6-phosphatase activity [*57,61,97*]. In keeping with previously discussed interpretations, these findings suggest that this enzyme adapts to increased metabolic turnover in the thyrotoxic state, and to the increased hepatic glucose production in the animal treated with growth hormone [*3,40*]. An increase in glucose-6-phosphatase activity has also been reported in mice bearing corticotropin-secreting tumors [*136*].

On the other hand, metabolic states presumably associated with decreased hepatic glucose production are accompanied by decreased glucose-6-phosphatase activity, as is the case in insulin-treated normal rats and to a greater degree in insulin-treated adrenalectomized rats [*8*]. The hypoglycemia-producing sulfonylureas decrease hepatic glucose production [*4,11,13,20,123*], and with protracted administration also decrease glucose-6-phosphatase activity [*10,37,65,84,152*].

There is evidence that fetal liver from the guinea pig lacks glucose-6-phosphatase [*106*] and that this enzyme is first demonstrable when the placental glucose infusion is interrupted by birth. Persistence of this fetal state has been suggested as a possible pathogenic mechanism in the classic form of glycogen storage (von Gierke's) disease [*36,62*], although studies on normal *human* fetal liver have revealed hepatic glucose production prior to birth [*153*]. Nevertheless, the documented absence of this enzyme in the glycogen storage syndrome very adequately explains the combination of excessive glycogen reserve with hypoglycemia, since in the absence of glucose-6-phosphatase, glucose-6-phosphate, once formed, can only be converted to glycogen or catabolized. The liver in glycogen storage disease retains another metabolic characteristic reminiscent of fetal liver metabolism, namely the ability to glycolyze rapidly. Whereas intravenous galactose administered to the normal child is rapidly cleared by the liver and converted to glucose or deposited as hepatic glycogen, in the child with von Gierke's disease galactose is almost quantitatively glycolyzed to lactate, resulting in severe metabolic acidosis [*132*].

Weber and Cantero [*157,159*] have found that glucose-6-phosphatase is absent in spontaneous and induced hepatomas of the rat. Thus, the resulting metabolic derangement in hepatoma

tissue is analogous to that observed in fetal liver and in the liver in von Gierke's disease. Metabolic studies on these tumors of the liver have demonstrated an inability to produce glucose [*15*], and in addition, assays for fructose-1,6-diphosphatase have failed to reveal significant activity of this enzyme [*160*]. These enzymatic findings are in agreement with the rapid glycolytic rates observed and the failure of this tissue to exhibit gluconeogenesis; i.e., conversion of labeled pyruvate to glucose [*15*].

EMERGENCY RESPONSES

The effects of dietary changes, fasting and hormones upon hepatic glucose production which have been discussed so far have mostly concerned rather "chronic" or "sluggish" effects occurring within several hours or longer. The liver, as the only major source of non-alimentary carbohydrate, must also respond in emergency situations with rapid glucose production.

Epinephrine. The hyperglycemic action of epinephrine has been known for more than half a century, but only recently have the enzyme mechanisms involved been identified [*115,116*]. By a complicated series of reactions epinephrine increases the activity of phosphorylase, the enzyme catalyzing the interconversion of glucose-1-phosphate and glycogen. (Fig. 4.) The mechanism whereby this change in enzyme activity invariably results in rapid glycogenolysis is unexplained. An increase in the concentration or activity of an enzyme should facilitate the attainment of a given equilibrium and thus accelerate the reaction in either direction, as dictated by starting conditions. However, the reaction may appear to be unidirectional when under the influence of the other adjacent enzyme systems of the intact cell. If so, glycogen synthesis may occur by another pathway, as has indeed been suggested [*18,28,107*]. Recently Leloir and Cardini [*87*] have reported incorporation of glucose-1-phosphate into glycogen in liver preparations which require uridine diphosphoglucose as a cofactor. Such a mechanism, if confirmed and shown to be physiologically significant, would indeed provide a route for glycogen synthesis not involving phosphorylase. If equilibrium conditions always favored glycogenolysis by phosphorylase, an increase or decrease in the activity of this enzyme would primarily control the rate of glycogen breakdown, as the facts seem to indicate.

Rapid increase in phosphorylase activity after the administration of epinephrine results in almost immediate glycogenolysis, increasing intracellular glucose-6-phosphate concentration which in turn leads to a rapid output of free glucose in the presence of glucose-6-phosphatase activity. It should be pointed out that glucose-6-phosphatase activity is not altered under these conditions, suggesting that this enzyme is not as a rule rate-limiting *per se*, but is kept in balance by its relation to glucokinase activity. A further factor which might be operative during epinephrine action relates to the non-competitive inhibition of glucokinase by the product of its activity, glucose-6-phosphate [*38,161*]. Any epinephrine-induced glycogenolysis leading to increased glucose-6-phosphate concentration would be expected on this basis to decrease glucokinase effectiveness, thus allowing glucose-6-phosphatase to predominate, and thereby enhancing hepatic glucose production.

Glucagon. The administration of glucagon, similar to epinephrine, leads to increased activity of hepatic phosphorylase within seconds, and also to increased production of glucose which, as in the case of epinephrine, is not accompanied by immediate changes in glucose-6-phosphatase activity [*27*].

Sodium and Potassium. Another mechanism for rapid stimulation of hepatic glycogenolysis has been partially elucidated [*28*]. Liver slices from a fasted rat show no net synthesis of glycogen from glucose or pyruvate when incubated in a medium containing high concentrations of sodium ion. When similar slices are incubated in a high potassium medium, net glycogen synthesis is readily obtained [*64*]. Likewise, liver slices from fed animals exhibit more rapid glycogenolysis when incubated in a high sodium medium compared to those incubated in a high potassium medium, and phosphorylase activity, when assayed, is found to parallel the rate of glycogenolysis [*28*]. Thus sodium ions, once they enter the intracellular fluid of the liver cell, appear to increase phosphorylase activity and thereby accelerate glycogenolysis. The liver cell is peculiarly sensitive to anoxia or trauma, and the slightest disturbance of its environment permits a rapid exchange of intracellular potassium for extracellular sodium [*43,49*], resulting in accelerated glycogenolysis by the increased phosphorylase activity until the cell membrane is again able to maintain normal ion gradients. When the intracellular environment is reconstituted with

potassium as the intracellular cation, phosphorylase activity falls to normal and glycogen synthesis can again proceed. This ion mechanism is therefore identical with the epinephrine effect in stimulating hepatic glucose production at times of stress, although its action is exerted at the cellular level directly.

SPECULATIONS

The dependence of hepatic glucokinase activity on insulin and the association of hepatic glucose-6-phosphatase activity with increased hepatic glucose production have been discussed. Since the increase in glucose-6-phosphatase activity follows other metabolic events, it is suggested that this enzyme adapts to increased substrate concentration [*78*]. However, the finding of decreased glucose-6-phosphate concentration in diabetic livers [*130*] argues against this explanation. Another objection is the recent finding of Freedland and Harper [*52*] that glucose-6-phosphatase returns to normal levels in animals maintained on prolonged low carbohydrate (high protein or high fat) diets. They state that perhaps the animal is able to adapt in other ways to the deficient diet and does not need to convert as much precursors to glucose as does the acutely deprived animal. One possible explanation for this late return of glucose-6-phosphatase activity to normal levels would be a concomitant prolonged and progressive fall in glucokinase activity over several days, as reported by Spiro [*142*] in diabetic rats acutely deprived of insulin. Thus glucose-6-phosphatase may be in relative excess compared to glucokinase and the balance of the activities of the two enzymes may continue to favor net hepatic glucose production. A recent report has suggested that direct synthesis of glucose-6-phosphatase occurs in fasting states since its increase in activity is inhibited by the administration of ethionine [*53*].

If the effect of insulin on glucokinase activity (as measured by the rate of glucose phosphorylation) is a direct effect of the hormone, insulin would then have two mechanisms of action, one enhancing hepatic glucokinase and the other altering cellular permeability in peripheral tissues. It is possible that at a subcellular level insulin exerts one and not two effects. In liver some intracellular membrane or interphase might be altered by insulin in a manner similar to its effect on the cell membrane of peripheral tissues. However, the sluggish response of liver compared to the almost immediate response of muscle is inconsistent with this unified hypothesis.

SUMMARY

A graphical summary of those aspects of carbohydrate metabolism herein discussed, which contribute to the regulation of blood glucose under normal and pathological conditions, is presented in Figure 9. The rate-limiting steps are drawn in a schematic liver cell (A). The normal animal with a blood glucose concentration of 100 mg. per cent (B) slowly mobilizes glycogen to increase the concentration of glucose-6-phosphate (G6P) which in turn is depleted by the prevailing, relatively low level of blood glucose; thus glucose is produced by the liver. At 150 mg. per cent (C) there is no net flow of glucose into or out of the liver since both glucokinase (1) and glucose-6-phosphatase (2) are respectively metabolizing glucose and glucose-6-phosphate at the same rate. At 200 mg. per cent (D) glucokinase (1) phosphorylates more glucose molecules than are cleaved by glucose-6-phosphatase (2), the concentration of glucose-6-phosphate increases and glycogen is deposited. Thus glucose is cleared by the liver.

In prolonged fasting states (E) glucokinase (1) falls by 50 per cent and glucose-6-phosphatase (2) increases. Thus glucose production is facilitated without a marked fall in blood glucose concentration. Intracellular glucose-6-phosphate concentration falls, effecting mobilization of glycogen. Fructose-1,6-diphosphatase activity (3) also increases and precursors are mobilized from the periphery under the influence of the adrenal cortex. In the diabetic liver (F) glucokinase is markedly decreased and glucose-6-phosphatase (2) is doubled. In spite of the alteration of both enzymes in favor of hepatic glucose production, the circulating hyperglycemia and the large inflow of precursors are able to keep the concentration of glucose-6-phosphate near normal, and therefore this animal has near normal glycogen reserves. In the adrenalectomized-diabetic animal (G), the supply of precursors is interrupted. Glucokinase (1) continues at the diabetic level, but glucose-6-phosphatase (2) is diminished to near normal values. Nevertheless, since the latter is still in relative excess, the concentration of glucose-6-phosphate is markedly reduced and is reflected by the limited reserve of hepatic glycogen.

Adrenalectomy in the animal with normal islet tissue (H) reduces the ability to mobilize

FIG. 9.

precursors from peripheral tissues and hence to supply them to the liver. Glucokinase (1) and glucose-6-phosphatase (2) remain relatively normal. Thus this animal can function in normal carbohydrate balance as long as adequate dietary carbohydrate is available, but is incapable of prolonged fasting since its only source of blood glucose is the limited supply of hepatic glycogen. The "steroid diabetic" (1) exhibits increased gluconeogenesis from peripheral precursors. Since there is adequate insulin, glucokinase (1) remains normal. Glucose-6-phospha-

tase (2) and fructose-1,6-diphosphatase (3) are increased. The marked difference between glucokinase and glucose-6-phosphatase characteristic of the diabetic state is not present in this animal, and the tremendous inflow of carbohydrate due to gluconeogenesis increases the concentration of glucose-6-phosphate, resulting in an extreme degree of glycogen deposition.

Exposure of the liver cell to epinephrine or glucagon (J) increases phosphorylase activity (4) which in turn causes a rapid breakdown of glycogen to glucose-6-phosphate. As a result of the increased glucose-6-phosphate pool free glucose is rapidly produced by glucose-6-phosphatase action. Since glucose-6-phosphate is also a non-competitive inhibitor of glucokinase, the relative activity of glucose-6-phosphatase is increased. Entry of sodium ions into the liver cell due to trauma or anoxia also activates phosphorylase and results in the same metabolic pattern.

Glucose-6-phosphatase (2) is absent in the liver cell of the child with glycogen storage disease (K) and consequently there is no glucose production. Lactate is produced when carbohydrate uptake exceeds its metabolism or the ability to deposit more glycogen. Hepatoma (L) has such a rapid glycolytic rate due to the absence of fructose-1,6-diphosphatase (3) that once glucose is phosphorylated it is rapidly metabolized to lactate. Glucose-6-phosphatase (2) also is absent in this tissue.

In conclusion, it would appear that hepatic glucose metabolism is not limited by cellular permeability as is the metabolism of glucose in peripheral tissues, but is largely controlled by the activities of the enzymes concerned with the entry and release of glucose into and from the pool of metabolic intermediates. Three separate mechanisms appear to be involved. The first is concerned with the routine day-to-day deposition of glycogen postprandially and its release between meals, and is associated with no major change in enzyme activity. The second mechanism is concerned with sustained alterations of enzymatic levels or activities secondary to metabolic changes of the animal. These alterations occur in diabetes, prolonged fasting states, as a result of special diets, adrenalectomy, and like conditions. They condition the establishment of that blood glucose concentration whereby a steady state with glucose-6-phosphate is obtained. The third mechanism effects immediate glucose production through the medium of glucagon, epinephrine or intracellular entry of sodium ions, as a result of rapid glycogenolysis secondary to a rise in phosphorylase activity.

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Seminar on Connective Tissue

Genetic Factors in Diseases of Connective Tissue*

A Survey of the Present State of Knowledge

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In recent years the concept of the connective tissue as an organ, or better, as a system, has gained general acceptance [*171b*]. The anatomy, physiology and chemistry of the connective tissue system is being subjected to intensive study. Disease of the connective tissue system has likewise been under increasing investigation.

An analysis of the role of genes in determining disorders of any system tends to divide naturally into two parts. First, there are less commonly occurring disorders in which a single mutant gene is primarily responsible for derangement in structure or function of the system under study or a part thereof. These disorders often have a theoretical import out of proportion to their clinical frequency because of the light they can shed on the biology of the particular system.

In the connective tissue system, the first category is made up of disorders such as the Marfan syndrome, the Ehlers-Danlos syndrome, osteogenesis imperfecta, pseudoxanthoma elasticum and the Hurler syndrome.

A second large category in which analysis of genetic factors is in order comprises the common diseases of the system. These are often diseases of multifactorial causation; in many the etiologic and pathogenetic mechanisms are as yet incompletely worked out; however acquired, or environmental, factors seem from existing evidence to be of paramount importance. None the less, genetic factors in determining susceptibility and in modifying behavior of the disease are evident in some. In the case of the connective tissue system, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, rheumatic fever and possibly others are illustrative of this second category.

After this major dichotomy one is left with "inborn errors of metabolism" such as gout and alcaptonuria, which involve the connective tissue system in such a striking and predominant manner that exclusion from a discussion of genetic factors in diseases of the connective tissue is out of the question.

In the field of the common diseases of connective tissue much information is currently in the process of collection. In each of the rare heritable disorders of connective tissue, more precise information on the nature of the basic defect is likely to become available in the near future. All this review can do is indicate the present status of a subject which one can hopefully expect to be advanced considerably in the next few years.

Part I

GENERALIZED DISORDERS OF CONNECTIVE TISSUE OF PRIMARY HERITABLE NATURE [*97*]

The Marfan syndrome is manifested by changes in the eye, especially ectopia lentis (subluxation of the lens); in the skeleton, especially excessive length of the round bones of the extremities; and in the aorta, especially a weakness of the aorta leading to diffuse aneurysm, dissecting aneurysm or a combination of the two types. Hernia and loose-jointedness are often conspicuous. One or more of the major components of the syndrome may be missing in a person who by evidence derived from the family and by the presence of at least one of the components almost certainly has the gene for the Marfan syndrome.

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The pedigree data are consistent with transmission of the Marfan trait as an autosomal dominant. The rather wide range in grade of severity of manifestations is consistent with dominant inheritance. Apparently sporadic cases, at least some of which may represent new mutations, occur rather often. Occasionally multiple affected sibs are the children of apparently healthy parents who are unrelated.

There can be little doubt that the Marfan syndrome is a generalized disorder of connective tissue. In what element of connective tissue* does the gene-determined defect reside? The dramatic changes in the aorta and the associated pathologic changes might suggest an innate defect of the elastic fiber. However, the suspensory ligament of the lens resembles collagen more than elastin [*104*]. Furthermore, there is more collagen than elastin in the aorta [*104*]. Loose-jointedness and hernia are to be expected, but how does a connective tissue defect lead to excessive longitudinal growth of bones? (Incidentally, the ribs appear to participate in the excessive longitudinal growth with resulting pectus excavatum, pectus carinatum, or an asymmetrical form of chest deformity. The round bones are not only excessively long but tend also to be abnormally slender.) One gets an impression that longitudinal growth is unreined. One possibility is that the connective tissue defect in the periosteum is responsible. Normally the periosteum, by its connections to the epiphyses, is dragged along the surface of the elongating bone and seems to exercise some control over longitudinal growth.

The predominant localization of aortic change to the ascending aorta requires explanation since the same defect probably extends throughout the aorta. It is to be noted that the aortic changes are not of the nature of a static congenital malformation. Instead, progressive changes occur, starting sometimes in the first year or so of life, sometimes not until considerably later. It is likely that hemodynamic factors peculiar to the ascending aorta determine predominant expression of the genetic defect at that site. Specifically, the ascending aorta is subject to much greater expansile pulsation with each heart beat than is any part of the arterial system beyond the aortic arch. "Structural fatigue" in the innately defective aorta is likely to occur.

The Ehlers-Danlos syndrome in full-blown form is manifested by changes in the joints, particularly hypermobility; in the skin, by unusual stretchability, ordinarily without loss of elasticity, fragility, bruisability, and peculiar scarring; and internally, by diverticula of the gastrointestinal tract, hiatus hernia, eventration of the diaphragm. In milder form the disease is difficult to diagnose with assurance. Hypermobility of joints and stretchability of skin are graded characters. There may be an entirely distinct entity of simple joint hypermobility which is genetical in etiology but determined by a different gene or genes.

Pedigree information is consistent with transmission of this trait as an autosomal dominant.

On first thought this disease might seem to represent a superabundance of elastic fibers. Indeed, this view has been held by some and finds support in the frequent although not uniform discovery of an excessive number of elastic fibers in biopsy specimens of subcutaneous tissue. However, it is probably the collagen elements of a joint capsule and of the skin (and, incidentally, also of a blood vessel) which determine how far the structure can be stretched. Elastic fiber elements function largely in the restoration to original position. The theory of Jansen [*77*] is particularly attractive: The defect in the Ehlers-Danlos syndrome involves the fasciculation of collagen fibers or at least the weaving of collagen bundles into a normal wicker-work. The collagen wicker-work appears in microscopic preparations of fresh tissue, in the Ehlers-Danlos syndrome, to be abnormally loose. The hyperplasia of elastic fibers which is sometimes found may be a secondary phenomenon; it seems from observations in tissue cultures that stretch is a stimulus to elastofibrogenesis. The demonstration of increased serum elastase-inhibitor in two patients with the Ehlers-Danlos syndrome [*171b*] may have important implications relative to the basic defect.

Osteogenesis imperfecta (OI) is not simply a disease of bone, although brittle and soft bones are usually the most impressive and important feature. Not only is the organic matrix of bone defective but also the collagen of sclera, leading to thin "blue sclerotics," of skin which is thin

* Bacchus [*7*] finds diminished serum "mucoprotein" in affected members of one family with the Marfan syndrome. Sjoerdsma and colleagues [*143a*] find increased urinary excretion of hydroxyproline, an amino acid unique to collagen, in some cases. Because of possible fundamental as well as diagnostic implications, confirmation will be awaited with great interest.

and produces broad surgical scars, of fascia leading to hernia, of ligaments, tendons and joint capsules leading to loose-jointedness. The deafness, usually referred to as "otosclerosis," appears to result from the same connective tissue defect in the bones and soft tissues in and around the middle and inner ear.

Histochemical studies [*42*] suggest (but do not prove) that collagen is qualitatively abnormal and not just insufficient in quantity. The experience with sickle cell anemia might suggest that an aberrant species of collagen is produced in OI and that its anomalous physical properties are responsible for the manifestations of the disease. To date studies have failed to delineate the possible molecular abnormality in this disease.

Classically, OI is transmitted as an autosomal dominant. Large pedigrees have been reported. There tends to be considerable variability in degree of severity between affected members of the same family and rather more between unrelated families. The possibility of an autosomal recessive form of the disease has not been excluded, especially for the severe form of the disease seen in the still-born or newborn, and referred to as osteogenesis imperfecta congenita. It is more likely, however, that individuals with the most severe cases of the so-called congenital type, born of normal parents, are heterozygous for a new dominant mutation. The birth of more than one such infant to normal parents is unusual. Genetic analysis is confused by the lack of specific diagnostic criteria in a disease which may well be heterogeneous.

Pseudoxanthoma elasticum (PXE) is by nature an abiotrophy. Although the fundamental defect of connective tissue is undoubtedly present from the beginning, the deterioration which makes its presence clinically evident does not occur until a few years or even a few decades after birth. The skin of the flexoral areas—neck, axilla, groin, and the like—becomes lax, grooved, ridged and slightly nodular. Crazing of Bruch's membrane behind the retina expresses itself as angioid streaking detected by funduscopy. The media of arterioles of intermediate and smaller size also undergo degeneration with premature calcification, peripheral arterial insufficiency, angina pectoris, hypertension, and especially a proneness to gastrointestinal hemorrhage. The collaboration of the innate defect and "wear-and-tear" is apparent in the pathogenesis of the skin and arterial changes.

Pedigree evidence is consistent with the transmission of PXE as an autosomal recessive.

Histological studies of skin, endocardium and small blood vessels show fragmentation of connective tissue fibers with resulting granular and irregular-shaped bits which tend to stain with "elastic stains" and have a pronounced affinity for the calcium ion.

The early view was that PXE represents an abiotrophy of elastic fiber—hence, "elasticum." A recent view is that the primary fault resides in collagen, that the elastic staining property is only mimicry, that PXE is a gene-determined variety of "elastotic degeneration of collagen." Even more recently, the counterattack on the collagen theory has been led by Rodnan and colleagues [*125*]. The matter of the basic defect must, in my opinion, be considered unsettled.

As to definition of the basic defect, *the Hurler syndrome*, or gargoylism, is in a more satisfactory state than any of the other heritable disorders of connective tissue. (There remain, to be sure, many details to be filled in.) The Hurler syndrome is a disorder of mucopolysaccharide metabolism. Chondroitin sulfate B and heparitin sulfate are excreted in the urine in considerable quantities and are found in large amounts in several tissues which have been analyzed [*105*]. Although the excreted and deposited mucopolysaccharides appear to be identical with those found normally in certain tissues, they are not excreted in the urine in normal persons, in appreciable quantity.

Related to the derangement in mucopolysaccharide metabolism, the skeleton is malformed in a characteristic and rather grotesque manner and changes in the soft tissue lead to stiff joints—claw hand and reduced mobility in most of the large joints. Excessive mucopolysaccharides are deposited in the liver, spleen and other viscera. Heavy deposits in the intima of the arteries lead to angina pectoris and sudden death, even in children a few years of age. The heart valves are defective, probably partly as an element of the connective tissue disease, partly as secondary damage from intimal deposits. The mucopolysaccharide can sometimes be stained in the leukocytes. The cornea is clouded in many cases. Impairment of intellect, one of the leading features, is progressive. The mechanism is not clear. The meninges become laden with mucopolysaccharide and internal hydrocephalus occurs in many cases. This is clearly not the entire

story, however. Heparitin sulfate may be toxic to the brain [*104*].

There are two genetic varieties of the Hurler syndrome (two genotypes) and there are phenotypic differences corresponding to the genotypic differences. More frequent is the autosomal recessive form of the disease affecting males and females equally and tending to show an increased rate of consanguinity among the parents. About 90 per cent of the subjects with this genotype have clouding of the cornea. The second variety is a sex-linked recessive,* like classic hemophilia. In this case, in which only males are affected, the cornea is probably never involved and the evolution of the disease is slower, so that, matched age for age, the patients with this form are as a whole less severely affected than are the patients with the first form. To the great surprise of many of us, no biochemical difference between the two genotypes, as to excretion pattern or tissue deposits, could be demonstrated.

Why the apparent excessive production of apparently normal mucopolysaccharides? Meyer and colleagues [*105*] believe that, whereas fibroblasts normally differentiate into several races, each producing only one mucopolysaccharide, "chemical metaplasia" of the fibroblast or disordered differentiation occurs in the Hurler syndome such that fibroblasts in many different tissues produce the two normal mucopolysaccharides in excess.

Most other conditions one could list, such as the various hereditary skeletal malformations, are not, by evidence now available, *generalized* disorders of connective tissue. *Fibrodysplasia* (formerly myositis) *ossificans progressiva* (FOP) does have features which may make it qualify as a heritable generalized disorder of connective tissue. The main feature is the progressive development of bone in fascia, aponeuroses and muscle sheaths. The back is first involved but eventually the extremities are affected as well, and almost all mobility of joints is lost. The thumbs and great toes are usually much stunted. It seems likely that this "malformation" of the first digits is due to a synostosis, with resulting monophalangy, which usually begins at least in utero but may be continuing in extra-uterine life and which is part and parcel of the fundamental perversion of ossification. The neck of the femur tends to be broad and short.

The basic defect may be an abnormality in the differentiation of fibroblasts such that those at the sites involved function as osteoblasts. An alternative possibility is that the connective tissue at the affected sites degenerates because of an innate defect or because of toxic effects from an unidentified metabolic derangement; ossification may be only a secondary phenomenon. I favor the first possibility.

FOP may be transmitted as an incomplete autosomal dominant, i.e., one with much reduced penetrance. This impression is based in part on the occurrence of some aspects of the syndrome, specifically dwarfed first toes and fingers, in successive generations with only occasional occurrence of the full syndrome.

The probably intimate inter-relationship of the non-cellular components of connective tissue is suggested by study of these hereditary disorders. In each, the defect has at some time or another been referred to at least two different connective tissue elements. In the Marfan syndrome the elastic fiber has been under suspicion. Collagen may in fact be defective and the recent preliminary findings relative to serum "mucoproteins" [*7*] suggest yet other possibilities. In the Ehlers-Danlos syndrome histologic evidence indicates derangement in both elastic fiber and collagen. It is even conceivable that a mucopolysaccharide is primarily defective and that a derangement of collagen fasciculation and elastic hyperplasia are secondary phenomena. Similarities in the behavior of collagen and elastin, particularly in degeneration, have been responsible for lack of agreement on the nature of the basic derangement in PXE. In the Hurler syndrome, before the evidence on a defect in mucopolysaccharide metabolism was assembled, collagen was under suspicion because of an abnormal histologic appearance. The experience with hereditary syndromes, therefore, supports the considerable body of evidence from other sources indicating an interdependence of connective tissue elements.

Clinical analysis, supplemented only by conventional pathologic studies, can achieve partial clarification of complex hereditary syndromes. The biochemist can be guided in his further investigation of the basic defect. Much remains to be done in the chemical characterization of the connective tissue in this group of diseases.

* It must be pointed out that when the disease makes procreation by affected males impossible, one may not be able to distinguish a sex-linked recessive from a sex-limited autosomal dominant with complete certainty.

Part II

RHEUMATOID ARTHRITIS*

Observations on the occurrence of multiple cases of rheumatoid arthritis in families [*34,47, 70,116,165*] would suggest a genetic predisposition. In 1950 the following was written [*93*]: "The relatively low incidence of a history of arthritis and the greater likelihood of the patients' remembering relatives affected with the disease as compared with the controls necessitates withholding any final conclusions on the matter until further investigations have been undertaken." As Short et al. [*140*] have stated: "Ordinary difficulties encountered in obtaining an accurate family history are enhanced by the confusion in the medical and lay nomenclature of the arthritides." Persons with a given disease are more likely to know of or remember the presence of the same disease in relatives than are "control" subjects who are free of the disease. Clearly the studies of most significance are those in which family members have been actually examined by as objective methods as possible. Selected ones of these family studies, most of them with controls, are presented in Table 1.

Rheumatoid arthritis has been studied in twins [*11,16,56*]. A higher rate of concordance for rheumatoid arthritis has been noted in monozygotic twins than in dizygotic. In Denmark, in a study still in progress, Thymann [*171*] found three cases of concordance in fourteen monozygotic twins and one case of concordance in fifteen dizygotic twins. The difference is not statistically significant. There was, however, an impressive similarity in the clinical behavior of rheumatoid arthritis in monozygotic twins: The onset was at the same age in two sets.

An increase in the frequency not only of rheumatoid arthritis but also of acute rheumatic fever has been found in the families of patients with rheumatoid arthritis in the past [*24,25,26, 29,34,134,*] and more recently by de Blécourt [*29*]. The latter study is particularly noteworthy because of its scope. In this survey conducted in Holland, about 7,000 relatives of 300 probands (100 with rheumatoid arthritis, 100 with ankylosing spondylitis, and 100 control subjects) were studied.

In any genetic study, especially when it deals with a condition which may not be fully "penetrant," that is, does not in all persons with the appropriate genetic make-up attain a level of expression which permits clinical recognition, a sensitive and specific test is an obvious desideratum. The hemagglutination test of the Waaler-Rose type seems to satisfy these requirements, in part at least, for rheumatoid arthritis. Lawrence and Ball [*88*] found a higher prevalence of rheumatoid arthritis in the families of seropositive probands than in the families of seronegative probands; they suggest that more than one distinct entity may be represented in what is clinically labelled rheumatoid arthritis. (Incidentally, no sex-difference in the prevalence of seropositivity was discovered.) Such studies must be controlled by subjects matched for age since there is a "growth curve" for sheep-cell positivity. The prevalence increases considerably at about age sixty-five. When they used a titer (in the proband) of 1: 32 as the dividing line between normal and abnormal they observed an increase in both x-ray and serologic evidence of rheumatoid arthritis in the family. Some seropositive individuals in families of patients with rheumatoid arthritis were free of clinical and radiologic evidence of rheumatoid arthritis. That the increased rate of seropositivity is *not* due to a common domestic environment is suggested by the failure of Lawrence and Ball to find any increase in the spouses of probands. The study in Leigh in Lancashire in 1950 had shown the same prevalence of rheumatoid arthritis in unrelated members of the households of persons with the disease as in control subjects.

Ziff and his collaborators [*190*] have also pursued a family study making use of a serologic test, Ziff's inhibition test [*189*], which gives almost 100 per cent positive results in cases of rheumatoid arthritis and about 4 per cent in others. A prevalence of 20 per cent positivity was found in first degree relatives* of patients with rheumatoid arthritis. A control group closely

* The gene-determined serum protein types discovered by Grubb [*57a*] and referred to as the Gm serum groups probably have no pathogenetic relationship to rheumatoid arthritis, although the serum of a patient with rheumatoid arthritis is used in the test. The Gm factor is a gamma globulin (hence the name) which inhibits the agglutination of Rh-positive human red cells of group O after coating with certain "incomplete" Rh (anti-D) antibodies. About 55 per cent of individuals have the gamma globulin. Although genetically determined and behaving as an autosomal dominant [*95a*], the Gm factor does not appear in the infant's serum until he is several months old; at birth the serum type of the infant is that of the mother, a finding which indicates placental transfer of the gamma globulin.

* Parents, sibs and children.

matched for age and sex showed 5 per cent positivity.

No satisfactory agreement with any simple genetic hypothesis has been demonstrated for family data on rheumatoid arthritis. Assuming monogenic dominant inheritance, Stecher et al. [*154*] had to postulate only 45 per cent penetrance for the gene. Assuming inheritance as a simple recessive, they estimate a penetrance of about 70 per cent.* On an assumption of simple recessive inheritance estimates of gene frequency have varied from 0.9 per cent (by Stecher [*154*] on the basis of an estimate of 0.58 per cent for the frequency of rheumatoid arthritis in the general population and an assumption of 70 per cent penetrance) to 36 per cent (by Lawrence and Ball [*88*] on the basis of incidence of 13 per cent positivity for the sheep cell agglutination test in relatives (of control subjects) over sixty-five years of age). The wide divergence of these values is an indication of the unsatisfactory state of the formal genetics of rheumatoid arthritis.

If inheritance of rheumatoid arthritis is as a simple autosomal recessive and if the frequency of the gene for rheumatoid arthritis is high, no great increase† in consanguineous matings is to be expected in the parents of affected persons. For the same reasons one may not be justified in rejecting the recessive hypothesis on the basis of the result of cousin marriages, as was suggested by Whittinghill and Hendricks [*178*] after studying a large kindred with rheumatoid arthritis. In this large North Carolina family the total number of individuals studied has swelled to 3,000 [*179*]. (Table 1.) There was no evidence of an alliance between rheumatoid arthritis and ankylosing spondylitis. Juvenile multiple rheumatoid arthritis‡ seemed to be independent of rheumatoid arthritis but related to ankylosing spondylitis [*179*].

* Stecher et al. [*154*] pointed out that by juggling penetrance estimates one could conclude either that rheumatoid arthritis is inherited as an autosomal dominant or that it behaves as a recessive. Neel [*110b*] also has pointed out the dangerous circular reasoning which may result from careless use of the concept of penetrance. A perfectly valid concept in a restricted usage, penetrance refers to what the geneticist sees. Non-penetrance merely results from inability to detect any phenotypic effect of the genetic background which from collateral evidence is believed to be present.

† De Blécourt [*82c*] found no increased prevalence of rheumatoid arthritis in the populations of Dutch islands with an increased frequency of consanguineous marriages.

‡ The results of family studies of "Still's disease," now in progress, will be awaited with great interest.

Although the role of heredity is difficult to quantify, it is noteworthy that the familial aggregation for rheumatoid arthritis was the only detectable difference in a study with matched control subjects performed in England and reported in 1950 [*93*]. All types of infections, accidents, inclement weather, faulty nutrition, unhygienic living and working conditions, pregnancy and its complications, menopause, social disaster, emotional stress and other factors were examined.

Psoriatic Arthritis. Although one would expect that two relatively frequent disorders such as typical rheumatoid arthritis and psoriasis would occur together by coincidence, with a frequency which is the product of the separate frequencies of the two diseases, there appears to be a distinct entity of arthritis specifically related to psoriasis. The finding of a negative sheep cell agglutination test in such cases, as well as clinical peculiarities, strengthens the impression. Mutilating involvement of the terminal phalanges, with progressive whittling away of the bone, is one rather characteristic feature [*138*]. Relationship between onset, severity and topography of nail and joint change seems to be stronger than that between skin and joint changes [*187*]. Most marked changes are, for example, likely to occur in the nail of the finger with most marked change in the terminal phalanx. Some [*53,135*] think ankylosing spondylitis in patients with psoriasis is an entity distinct from the common type.

Assuming that psoriatic arthritis is one facet of the psoriasis syndrome which although not present in all cases with skin involvement has the same genetic background, the remainder of the discussion in this section will concern genetic studies in which the psoriatic skin lesion was the trait investigated. (A thorough study of arthritis in psoriatic families has not, to my knowledge, been done.) There is clearly a strong hereditary influence in the pathogenesis of psoriasis [*6,73*]. Lerner [*91*] "is of the opinion that any research of the familial incidence* of psoriasis which

* Ingram [*73*] reported that 33.9 per cent of patients have a positive family history and Church [*23*], in a population also drawn from Yorkshire, stated that 34 per cent were familial. This manner of expression should be discouraged. It means relatively little and creates a false impression of quantitation. It means little because as pointed out by Steinberg [*158*], with apologies to Gilbert and Sullivan, one does not know how many of "his sisters and his cousins and his aunts" were investigated or how many of them were medically well known to the proband-informant.

TABLE 1

Investigator	Rheumatoid Arthritis (RA)	Controls	Remarks
Lewis-Fanning (1950)	N = 532 (c̄ RA) Incidence of RA in fathers 7% mothers 15% sibs 3.8% (n = 2,151)	N = 532 (s̄ RA) 3% 9% 1.8% (n = 2,143)	.....
Barter (1952)	RA in $^{59}/_{538}$ relatives (11%) fathers 5% mothers 13% brothers 8% sisters 15%	RA in $^{35}/_{543}$ relatives (4.6%)	.....
Miall (1955)	Parents and sibs of 59 males with RA—3%	0.6% RA in randomly selected controls	.....
Short, Abrams and Sartwell (1952)	N = 293 (c̄ RA) 11.9% + F.H. for RA	N = 293 (s̄ RA) 5.1% positive F.H.	Difference in percentages 6.8 ± 2.3
Short, Bauer, and Reynolds (1957)	11.6% + F.H. for rheumatic fever 23.2% + F.H. for "undetermined and other forms of rheumatism" 5.8% + F.H. for symptomatic degenerative joint disease	3.4 12.6 5.1	Difference 8.2 ± 2.2 10.6 ± 3.2
Stecher, Hersh, Solomon and Wolpaw (1953)	N = 224 (c̄ RA) 3.1% RA (n = 1453) all relatives 5% RA—relatives over 50 yr. of age	N = 448 (s̄ RA) 0.58% RA (n = 2311) 0.9% RA (over 50 yr.)	.....
De Blécourt	N = 100 In 2,000 relatives prevalence varied from 0.6% in male cousins to 10.0% in mothers average 2.6%	N = 100 0.2% 3.0% 0.8%	.....
Whittington et al	Of 28 RA cases in kindred of 3,000 persons 1° relatives 3.7 ± 1.14% 2° relatives 2.4 ± 0.64% 3° relatives 2.3 ± 0.60% $^{19}/_{28}$ had at least one affected relative in three degrees of relationship.	.....	.....

yields less than one-third of familial cases should be regarded as inadequate."

The rarity of psoriasis in the Orient may have its basis, in part, in genetic differences. Lomholt [*96*] in a detailed survey of psoriasis in the Faroe Islands found 312 cases in a population of 11,000 (2.84 per cent). She considered the trait to be dominant with incomplete penetrance.

Pfändler [*114*] reported monozygotic twins concordant for psoriasis and found in the literature reports of sixteen pairs of monozygotic twins of which eleven were concordant (both twins affected). Among twelve pairs of dizygotic twins only two were concordant. He suggested that a dominant gene with reduced penetrance is responsible for the disease. Hoede [*66*] found eleven per cent of siblings affected when one parent was affected and 4.5 per cent when both parents were unaffected. In three families with both parents affected, five of nine children were affected. In 1945 Romanus [*129*] reported the following figures for the incidence of psoriasis in the first degree relatives of index cases: parents 8.3 per cent, siblings over thirty years, 9.0 per cent; offspring over thirty years, 13.0 per cent.

The difficulties in distinguishing between a

TABLE II

X-ray Description	Number	Rheumatoid Arthritis
Nodular fibrosis (Caplan syndrome type)	20	55
Progressive massive fibrosis	59	3.3
Simple pneumoconiosis	92	0
Normal	112	0

recessive autosomal gene with high gene frequency and a dominant autosomal gene with reduced penetrance are well illustrated by this disorder. The studies to date [*91*] do not seem to permit a distinction. An even more complex genetics—two recessive genes, both of which must be present in homozygous state for expression of the disease— was postulated by Steinberg and colleagues [*155–157*].

The Caplan Syndrome. Over six years ago Dr. Anthony Caplan [*21*] of Cardiff noted in Welsh coal miners that rheumatoid arthritis was associated with a characteristic type of pneumoconiosis (see Miall [*106,107*] for a reproduction of the x-ray appearance). The characteristic lesion consists of multiple globular masses 5 mm. to 5 cm. in diameter with little fibrotic change in intervening lung. The lesions develop in crops and evolve rapidly. In this respect as well as in general appearance they suggest metastatic carcinoma. The changes in this nodular fibrosis of the Caplan syndrome are distinguished from those of progressive massive fibrosis [*41*]. PMF evolves more slowly against a background of appreciable "simple pneumoconiosis." The shadows in PMF are more likely to be irregular in outline and lead to more distortion of surrounding lung with emphysema. The changes of the Caplan syndrome are also distinct from the interstitial pneumonitis which may accompany rheumatoid arthritis without exposure to dust [*35*].

After Caplan had suggested from clinical impression that there is a characteristic pneumoconiosis in coal miners who also have rheumatoid arthritis, a survey was made of the incidence of rheumatoid arthritis in patients with various types of lung changes by x-ray or with a normal chest x-ray. These studies were carried out by the Epidemiologic Section of the Pneumoconiosis Unit at Cardiff, without knowledge of the x-ray findings. The criteria for the diagnosis of rheumatoid arthritis were characteristic history and characteristic x-ray changes and/or positive sheep cell test. The categories studied and the incidence of rheumatoid arthritis determined are shown in Table II. Over 50 per cent of patients with nodular fibrosis (Caplan type) showed rheumatoid arthritis. Even when rheumatoid arthritis was not present in the proband with the characteristic pulmonary change, the frequency of rheumatoid arthritis was increased in the family, suggesting that the genetic tendency was present in the proband even though it did not happen to be expressed. The Waaler-Rose hemagglutination test was found to be positive in the Caplan syndrome [*8*].

Pathologic studies of the lung lesions in Caplan's syndrome have been provided by Gough [*51*], Campbell [*19*], Caplan [*22*] and Rickards [*122*]. The ball-like lesions seen by x-ray are found to consist of alternating concentric layers of partially necrotic collagen and pigmented dust. To some extent palisading of "fibroblasts" is evident and at least a superficial resemblance to the subcutaneous rheumatoid nodule is produced. In slightly less than a half of the cases changes characteristic of tuberculosis have been identified in intimate association with the ball-like lesions. The tubercle bacillus is thought by the Cardiff group to be a contributing factor in many or most cases of the Caplan syndrome.

The over-all results "suggest an inherited abnormality of tissue reaction, not confined to the skeletal system." Both a "rheumatoid" genetic predisposition and exposure to coal dust seem to be necessary for development of the characteristic lung lesions. There was no evidence that exposure to coal dust was responsible for the rheumatoid arthritis; rheumatoid arthritis was equally frequent in the group of miners and ex-miners and the group of non-miners [*106*].

Cases of the Caplan syndrome have been described in coal and silica workers in many parts of Europe [*19,33,80,122*] and recently the first case has been reported from this country by Kantor and Morrow [*79*] of Wilkes-Barre, Pennsylvania. The syndrome has been described in a man with asbestos exposure [*122*].

Sjögren's Syndrome. This syndrome [*46,62b*] is characterized by keratoconjunctivitis sicca, dry mouth and mucous membranes (xerostomia, rhinitis sicca, pharyngolaryngitis sicca), symptoms of systemic disease and arthritis like rheumatoid arthritis. The parotids are usually

enlarged and show lymphocytic infiltrations on biopsy. The patients are mainly women past the menopause. The sheep cell agglutination test is usually positive when arthritis is present. Serum globulin concentration is often greatly increased and cryoglobulin may be present. An autoimmune basis for the disease has been suggested by the recent demonstrations of autoantibodies to salivary and lacrimal gland extracts [*78*]. Denko and Bergenstal [*30*] commented on the occurrence of "a strong positive family history." Falls [*39*] observed Sjögren's syndrome in two sisters; Coverdale [*25b*], in a man and daughter; Lisch [*95b*] in twelve individuals in three generations of one family. Further family studies may help clarify the nature of this puzzling entity.

ANKYLOSING SPONDYLITIS

Familial incidence was emphasized by both von Bechterew [*175*] and Marie [*99*] in their classic descriptions of the disease to which their names are sometimes assigned. Of von Bechterew's three cases, spondylitis occurred in the mother, sister and child of one, in the mother and aunt of a second, and the mother of the third. No one has disputed the importance of heredity [*14,86,101,112,127,169,177*].

Beyond the scope of this survey is a discussion of whether rheumatoid spondylitis and peripheral rheumatoid arthritis are the same disease [*140*]. It is, however, worthy of note that genetically the behavior of the two entities is different, as is evident from a comparison of this section with that on rheumatoid arthritis. Genetic behavior is often a nosologic aid in the delineation of distinct pathogenetic entities.

In any genetic analysis the diagnosis of the disease-trait under study must be carefully made. Otherwise, the inclusion of cases of fundamentally distinct conditions will confuse the results. Reference is made to publications [*14,135,136*] on the important differential diagnostic problems in ankylosing spondylitis.

As evidence of the heritability of ankylosing spondylitis can be quoted the description by Stephens and Nunemaker [*161*] of its occurrence in identical twins reared apart. Campbell [*18*] described the simultaneous onset of ankylosing spondylitis in monozygotic twins living in different countries. Rogoff and Freyberg [*127*] had a set of identical twins with ankylosing spondylitis, as did Ray [*119a*].

When the prevalence of a disease is found to decrease step-wise as the degree of relationship to the proband increases, evidence at least consistent with a genetical hypothesis is provided. One group [*179*] found in a single very large kindred the following frequencies of ankylosing spondylitis in relatives of spondylitic probands: First degree relatives 6.3 ± 2.48 per cent; second degree relatives 2.29 ± 1.16 per cent; third degree relatives 0.7 ± 0.5 per cent.

The leading genetic hypothesis relative to ankylosing spondylitis is that proposed by Stecher and his colleagues [*64*]: susceptibility may be determined by a single dominant autosomal gene which in the male is about 70 per cent penetrant* and in the female about 10 per cent. There is a suspicion [*54*] [*123*] that in the children of an affected woman penetrance is complete, or more nearly complete, in both men and women. In general, women are more often affected in the "familial" instances of ankylosing spondylitis than in the sporadic cases [*43*]. A lack of nosologic homogeneity is possible, or the influence of "modifying genes" may account for these observations.

Hersh, Stecher et al. [*64*] estimated that about six persons in every 10,000 have the gene for susceptibility to rheumatoid spondylitis.

The description [*102,111,128*] of a high incidence of prostatitis and seminovesiculitis† in male patients with ankylosing spondylitis requires evaluation. From the point of view of medical genetics the concept of a collaboration between a genetic factor for ankylosing spondylitis and an acquired factor such as genitourinary infection is an agreeable one. The increased "penetrance" of the genetic factor in males as compared to females could find its explanation in such a collaboration. Genitourinary tract infection and the gene for ankylosing spondylitis might be "necessary and sufficient causes"; both might be necessary and neither alone sufficient.

OSTEOARTHRITIS

Primary Generalized Osteoarthritis. What is diagnosed osteoarthritis seems to occur in multiple members of families more often than should occur by chance alone [*5,63,134,*]. The condition

* A penetrance figure of only 18 per cent for heterozygous males was calculated by Whittinghill and Hendricks [*178*].

† It is not Reiter's syndrome which is referred to, although Reiter's syndrome can be confusing because of associated spondylitis. Familial occurrence of the Reiter's syndrome is so rare [*170*] as to represent coincidence almost certainly.

may be transmitted as a Mendelian dominant but the details of the nosology in this complicated area and of the genetical dynamics are yet to be reported. Kellgren and Lawrence [*81,82a,82b*] suggest the existence in the large category of "osteoarthritis" of a specific "primary generalized osteoarthritis" for which there is a strong familial predisposition.

Obesity, a recognized basis for unusual osteoarthritis, is in part genetically determined [*22b*]. Osteoarthritis appears to be a disease of wear and tear on cartilage, and several conditions with genetic determination increase this wear and tear. Conceivably there are genetic variations in the susceptibility of cartilage to wear. It must be pointed out that in the majority of persons over forty years of age there is x-ray evidences of osteoarthritis in at least some parts of the skeleton.

Heberden's Nodes. Stecher [*149,152*] believes that Heberden's nodes are distinct from generalized osteoarthritis. Kellgren and Moore [*81*], however, thought they are one and the same disease. This controversy aside, the trait, Heberden's nodes, appears to be determined by a sex-influenced autosomal gene; it behaves as a dominant in the female, as a recessive in the male. Penetrance is essentially complete in white women over seventy years of age, that is, by that age nodes have developed in all women who carry the gene. About 27 per cent of women and 3 per cent of men over seventy years have the trait. After artificial menopause Heberden's nodes may develop in women as young as thirty-two years.

Hereditary Disorders Predisposing to Osteoarthritis. It is known [*41,52*] that certain clearly hereditary malformations of the hip predispose to osteoarthritic change in that joint at a later age. Included in this group are (1) congenital dislocation of the hip [*60,61*] and (2) Legg-Calvé-Perthes' disease of the hip (osteochondrosis of the femoral capital epiphysis).

Legg-Calvé-Perthes' Disease (LCP) occurs very rarely in Negroes. (Aseptic necrosis of the hip in sickle-hemoglobin C disease may simulate LCP.) Goff [*50*] claims there is a somatotype peculiar to LCP. Many of the reported pedigrees of LCP are outlined by Goff [*50*]. In some cases the inheritance has been that of a clear-cut dominant with essentially full penetrance, e.g., the family of Stephens and Kerby [*160*] in which an affected Welsh woman produced by two husbands descendant lines in which 27 of 120 individuals (in five generations) were affected. In other pedigrees [*31*] the heredity is less clear, but the trait may be dominant with varying expressivity. In some families multiple osteochondroses of epiphyses other than the hip have been observed [*1*]. The incidence of LCP in the white population of the United States is probably between 10 and 100 per 100,000 births.

Evans [*36*] followed up fifty-two cases of LCP at a time as long as thirty-six years after treatment; the average length of follow-up was sixteen years and the average age of the patients at follow-up was twenty-three years (oldest forty-two years). Results were good in fifteen, fair in twenty-one and poor in sixteen. Clinical results correlated closely with the x-ray judgment of the state of the femoral head. The results were poorest in girls and in those subjects in whom the disease started after the age of eight years.

Congenital dislocation of the hip occurs about five times more often in female offspring than in males [*25a*]. The dysplasia of the hip responsible for dislocation may be inherited as a sex-influenced autosomal dominant [*61*].* Idelberger [*72*] has reviewed the role of heredity in 16,343 cases reported in the literature. Even when there is little clinical evidence of the abnormality, roentgenograms may reveal an abnormally shallow acetabulum. Secondary traumatic aseptic necrosis from improperly managed dislocation may simulate LCP and lead to subsequent osteoarthritic change. Roentgenographic studies of members of the family of patients with dislocation of the hip often reveal multiple cases of acetabular dysplasia without dislocation. (Fig. 1.) Premature osteoarthritis tends to occur also in cases of the latter type. Putti [*116b*] thought as many as 40 per cent of cases of osteoarthritis of the hip have their basis in congenital dysplasia.

Coleman [*25a*] estimated that congenital dysplasia of the hip of the type which is responsible for dislocation occurs in at least mild form in about one of each 110 births. He has described an instructive case illustrating the role of congenital dysplasia in the development of osteoarthritis of the hip: A thirty-two year old woman in 1949 had skeletal roentgenograms taken be-

* Some [*119b*] suggest multifactorial inheritance. Hass [*61*] emphasizes that what he calls typical dislocation develops in extrauterine life, is anterior-superior in location and is hereditary. Genetic analysis may be confused if one fails to distinguish what Hass calls the teratologic type of dislocation in which posterior-inferior displacement has its onset in utero and a genetical factor is less clearly demonstrable.

cause of symptoms of spondylolisthesis. The hip showed mild extrusion of the head of the femur. Six years later the patient returned with moderately far advanced osteoarthritis. This disease is one in which preventive medicine can accomplish much by detecting the presence of hip dysplasia in the newborn and instituting proper therapy in the first days or indeed hours of life. Kemp [*83*] placed the incidence of "congenital dislocation of the hip" in Denmark at 100–150/100,000 births.

Familial epiphysial stippling, or epiphyseal dysplasia punctata [*121*], is another condition which may simulate an arthritis and may lead in later life to osteoarthritic change. The various chondrodystrophies, e.g., classic achondroplasia, probably also render the subject prone to osteoarthritis.

Jacobsen [*75*] described a seemingly sex-linked recessive variety of "osteochondrodystrophia deformans" in five generations of a single family. Two female carriers of the trait had "marked arthritic processes in the hips and ankles." Ullrich [*174*] suggested that the forty-seven and forty year old patients described in 1938 by Schmidt [*132*] as suffering from "a previously undescribed form of familial degeneration of the cornea in association with osteoarthropathy" and in 1927 by Schinz and Furtwängler [*131*] as "hereditary osteoarthropathy with recessive inheritance" might represent a mild ("late") variant of the Hurler syndrome.

Multiple epiphysial dysplasia [*103*] is a condition which may be confused with Legg-Perthes' disease. It is also distinct from dysplasia epiphysialis punctata. Fairbank [*39*] provided one of the earliest clear descriptions. (The adjective "multiple" is used advisedly in place of generalized.) Symptoms in the form of joint pain and stiffness begin in childhood. Hips and knees are especially likely to be symptomatic but ankles, hands, spine, shoulders and elbows have been affected in some reported cases. The patients tend to be short, but marked dwarfism is rare. The fingers are short and stumpy. The knees may be knobby from enlargement of the epiphyses. As Barrie et al. [*9*] state, "Osteoarthritis is an inevitable complication, beginning, as may be expected, in early adult life."

Familial Osteoarthropathy of the Fingers. Under this designation Allison and Blumberg [*4*] have given an excellent description of the type of avascular necrosis of the phalanges of the hands to which the name of Thiemann is sometimes

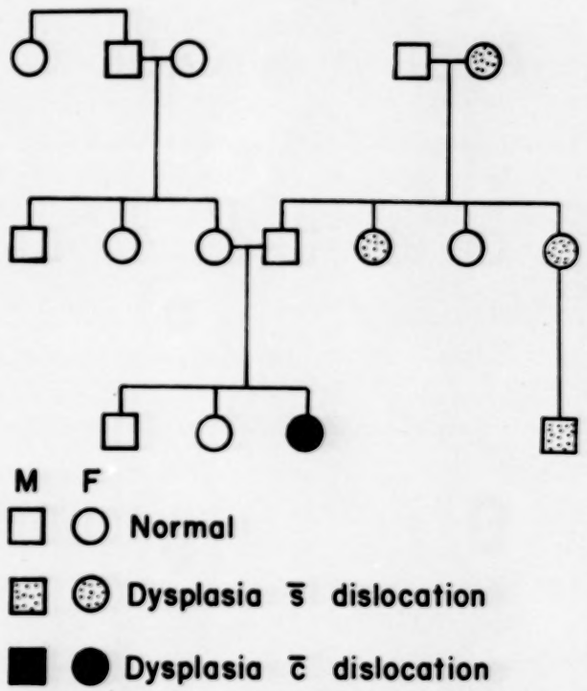

FIG. 1. The distribution of hip dysplasia, with and without dislocation, in a family (after Faber [*36*]).

attached [*137*]. It is inherited as an autosomal dominant with strong penetrance. A consanguineous mating between two affected persons resulted in two of six offspring who had more severe deformity in the hands than any of the others. These were thought to represent homozygous dominants. Painless deformity at the *proximal* interphalangeal joints begins in childhood or adolescence. There is no involvement of the rest of the skeleton in many cases, although in others what is indistinguishably the same disease is associated with Legg-Perthes' disease and other forms of epiphysitis [*1,15*].

SYSTEMIC LUPUS ERYTHEMATOSUS

A familial incidence of discoid lupus has been recognized for some time [*89,100,176*]. Multiple cases of SLE have been reported in the same family in a few instances [*115*].

Singer, Motulsky and Shonberge [*142*] observed a woman with SLE whose sib had thrombotic thrombocytopenic purpura. Davis and Gutridge [*27*] described SLE in identical twin sisters; Wagenhals and colleagues [*176b*] also reported on its occurrence in identical twin sisters. Glagov and Gechman [*49*] reported on SLE in a mother and daughter. McCuistion and Schoch [*98*] described possible discoid lupus in a newborn infant and the subsequent development

of SLE in the mother. Willis [*180*] reported on a sibship of six in which three sisters had died of typical SLE at ages ten, nineteen and twenty. The diagnosis was confirmed at autopsy in two. In two other sisters there was mild hypochromic anemia and splenomegaly. A brother was apparently normal, as were the parents. "LE-cell proved" SLE was described in two sisters by Agranat, Bersohn and Lewis [*3*]. Bloom [*13b*] had under observation a twenty-seven year old woman, with SLE, whose sister had died of the disease at age twenty-two. Zeisler and Bluefarb [*188b*] observed a sister and brother, the former with chronic discoid and the latter with subacute SLE.

Leonhardt [*90*] described a sibship with fourteen members in which six were normal, four had moderate elevation of serum globulin, and four had pronounced hyperglobulinemia. Three of the four with marked hyperglobulinemia had SLE. The parents had normal serum proteins and no SLE—a fact which weakens any suggestion that a recessive trait (with heterozygous state of the healthy sibs who had moderate elevation of serum globulin) is involved; the parents should, if this were true, be heterozygotic and show moderate elevation of globulin.

A biologic false positive serologic test for syphilis, sometimes an indication of otherwise inapparent SLE, was observed by Cannon [*20*] in the female but not the male members of a sibship. The factor responsible for the biologic false positive reaction is capable of placental transmission. The L.E. factor also displays placental transmission [*12,17,110a*]; both factors persist in the infant's blood for only a short time.

Unsuspected complexities of the immunologic inter-relationships of mother and fetus are continually being uncovered. A familial incidence of SLE might have its basis in this realm and not operate through genes. However, the occurrence of SLE in father and children might be difficult to explain by such a mechanism. *In summary:* The evidence for genetic factor(s) in SLE is at present meager. When the chemical defect in SLE is better defined, family studies can be conducted with possible profit.

RHEUMATIC FEVER

In only about 3 per cent of patients who have acute streptococcal pharyngitis does rheumatic fever subsequently develop [*117*]. May the genetic constitution of the individual determine whether he falls into the 3 per cent group? A crucial experiment would seem to be a comparison of the incidence of rheumatic fever in persons with and without a family history of rheumatic fever living under closely similar conditions. At least one study of this type [*118*] has been performed in a military setting. Of 122 individuals who had a history* of rheumatic fever in their immediate families and in whom a streptococcal infection developed, rheumatic fever developed in 3.3 per cent. This was not significantly greater than the rheumatic fever rate of 2.7 per cent in 1,359 "controls," the subjects in another series. (Of course, a proportion of the "controls" had strong family histories for rheumatic fever. Although the data do not support the genetic hypothesis, neither do they reject it.)

There are difficulties with such studies in that the age of maximum risk may be passed and in a group of recruits some selection has already been exercised. (Rammelkamp [*118*] thinks this objection may not be valid, witness the similar attack rates for rheumatic fever in these eighteen to twenty year olds as in children.) One would be most interested in first attacks.

Somewhat more indirect, yet convincing, evidence of the operation of genetic factors in determining susceptibility is available. Studies conducted in Baltimore [*48*], New York City [*182,183,185*], New Haven [*55*], Toronto [*173*], Belfast [*163,164*], Chicago [*32*] and elsewhere have demonstrated a familial aggregation of cases of rheumatic fever which probably is not explicable on the basis of common environmental factors. Some [*32,185*] interpret the findings as indicative of unifactorial autosomal recessive inheritance. Others [*55,164,173*], while supporting the genetic hypothesis, insist that the data are not consistent with any simple mode of inheritance.

The incidence of rheumatic fever in the members of sibships studied, that is, the proportion of affected children, varies, depending on whether both, one, or neither of the parents are affected [*163,185*]. This is a feature which a genetically influenced trait should display but by itself it does not prove the genetic influence.

Wilson's group [*182*] applied the *a priori* method for testing the recessive hypothesis and found satisfactory agreement. Furthermore, an analysis of the progeny of different types of matings categorized not only by whether the parents

* See footnote on p. 288 relative to the necessity for careful specification and quantification of positive family history in studies such as these.

themselves were or were not rheumatic but also by the presence or absence of rheumatic fever in close relatives of the parents likewise resulted in close agreement of observed incidence of rheumatic fever with the incidence predicted by the autosomal recessive hypothesis.

Alleged constitutional differences of persons susceptible to rheumatic fever [*58,68,71,85,92,141*] —fair skin, freckles, red hair— probably will not stand up under statistical scrutiny. As Paul [*113*] states, measurements in this field are very difficult. Hewitt and Stewart [*67*] thought there was no evidence of a familial aggregation which required genetic explanation but did find a significant excess of "non-blue-eyed" individuals with rheumatic fever. In fact, it was estimated by them that the risk of rheumatic fever was twice as great in "non-blue-eyed" as in "blue-eyed" persons.

Twin studies indicate a greater concordance for rheumatic fever in monozygotic twins than in dizygotic twins [*74,129,163,184*]. However, more extensive data and more precise determination of zygosity are desirable.

Presumably, genetic factors determine the qualitative and/or quantitative nature of the host response to repeated streptococcal infections. The mechanism of this gene action is, of course, of great interest but is unknown. Adams and colleagues [*2*] found that among the parents and siblings of children with rheumatic fever there was a significantly greater proportion with low serum concentrations of non-specific hyaluronidase inhibitor (33 per cent of 131) than in normal control subjects (10 per cent of 71).

Secondary prevention of rheumatic fever by long-term prophylaxis of individuals once attacked by rheumatic fever has been outstandingly successful but has some practical difficulties. Characterization of genetic susceptibility could permit *primary* prevention. Presently available evidence dictates particular vigilance with regard to streptococcal infection in children of families with a history of rheumatic fever.

Part III

GOUT [*167*]

A familial incidence of gout was noted before the changes in the level of uric acid in the serum was discovered, indeed before uric acid was discovered by Scheele (1776). In fact, a hereditary influence was commented on by Galen in the second century A.D. Sydenham clearly indicated the hereditary background [*144*]. Scudmore [*133*] early in the last century made a fairly systematic study of familial incidence; he found that 75 per cent of gouty patients had a history of the disease in a parent or grandparent. A statement requiring confirmation was made by Futcher [*44*] in 1914: "Although the women of gouty families may escape gouty manifestations, they are more likely to transmit the disease to their offspring than are the men. A grandson may inherit gout from a gouty grandfather through a mother who has never shown any manifest symptoms of the disease." None would dispute the latter statement; whether or not the first is true is uncertain.

In the last twenty years a number of studies have accumulated in which serum uric acid was used as the trait for study. Several groups of workers [*146–148,151,153,168,181*] have advanced the view that hyperuricemia is inherited as an autosomal dominant.

Another view favored by Hauge and Harvald [*62a*] is that the uric acid level is under multigenic control and, as a corollary, hyperuricemia is the result of a cumulative effect of several genes. The concept is that the uric acid level is a graded trait ("a continuous variable") like height or skin color. This view is an attractive one in the light of recent experiences with attempts to relate a particular objective measurement to a particular disease (e.g., blood pressure and hypertensive cardiovasculorenal disease; serum cholesterol and atherosclerotic cardiovascular disease) and to work out the inheritance of the objective measurement.

For blood pressure and serum cholesterol, and almost certainly for serum uric acid, the effects of race, age and sex must be accounted for before intrafamilial correlations are possible [*147*]. The scoring method used by Fraser Roberts [*57b*] for blood pressure can be equally well applied to uric acid provided extensive data on variations with race, age and sex are available. Such data derived from population studies we do not now have.

Another reason that one is attracted to the view that uric acid concentration is a continuous variable is that more than one metabolic step leading to uric acid, each under genic control, is known. Uric acid synthesis is probably under hormonal control, or at least hormonal influence, and the hormonal characteristics, even in one sex, vary, in part because of genetic differences. (Three groups [*69,124,186*] have reported a de-

creased 17-ketosteroid excretion in gout. Interpretations of these reports differ and further evaluation is needed.) In the third place, there is now conclusive evidence that uric acid can be broken down in man [*162*]. Therefore, genetic differences in this capacity are possible. Variations in renal clearance of uric acid are likely, although it now seems clear that the hyperuricemia of gout is not primarily due to this cause. Whatever the mechanisms, overproduction of uric acid seems to be responsible for the hyperuricemia of gout [*162,188*].

If the serum uric acid is sufficiently elevated for a sufficiently long period, then clinically evident gout is likely to develop. Whether or not there are other factors, for example characteristics of the connective tissue, which predispose further to the development of gout is unknown. Certainly hyperuricemia *per se* is not responsible for precipitating the acute attack of gout; other factors which may be partially under genetic control seem to be operating. The adrenocortical hormones may somehow be involved in the onset of acute attacks.

It remains to be determined whether or not clinical gout is more likely to occur in polycythemia, leukemia, pernicious anemia and uremia if the subject is a member of a gouty family.

ALCAPTONURIA AND OCHRONOTIC ARTHRITIS

For about fifty years, i.e., since Garrod, the basic deficiency in alcaptonuria has been presumed to involve the oxidation of homogentisic acid. Direct proof of a deficiency in the enzyme, homogentisic oxidase, has been provided by the group at the National Institute of Arthritis and Metabolic Diseases in Bethesda [*87*]. Use was made of liver tissue from an alcaptonuric patient who required abdominal surgery for repair of a hiatal hernia.

Ochronotic pigment derived from homogentisic acid is deposited in cartilage [*143a*] and connective tissues surrounding joints [*13,45*]. (Deposition in the pinnae and scleras has diagnostic implications.) Scarring and calcification seems to be incited in response to the deposition, although the details in the pathogenesis of the arthritis remain obscure. (Parenthetically, it is noteworthy that similar changes may be produced in the heart valves with resulting calcific aortic stenosis [*94*].) There are some similarities to osteoarthritis and possibly the ochronotic deposits predispose to the wear of cartilage which appears to be the primary step in that disorder.

Following the suggestions of Bateson, Garrod concluded that alcaptonuria is inherited as an autosomal recessive. Recently, dominant inheritance has been claimed [*108,109*] for some pedigrees. Knox [*84*], however, questions whether or not these are truly other than examples of recessive inheritance. Specifically, mating of affected individuals with heterozygous carriers may account for the finding of affected individuals in successive generations. This reviewer is convinced by Knox's argument that in accordance with the Hardy-Weinberg law the incidence of heterozygotes for alcaptonuria may be relatively high (about 1 in 200!) and the occasional mating of an affected person with such a heterozygote is to be expected. The more pedigree which are found to show involvement in successive generations, the greater is the probability that there is indeed a form of alcaptonuria which is transmitted as a dominant. However, conclusive evidence would be demonstration of a qualitative or quantitative biochemical difference between affected members of families which seemed to demonstrate different patterns of inheritance.

Part IV

OTHER ENTITIES

The Connective Tissue Disease Associated with Agammaglobulinemia. The hereditary form of agammaglobulinemia is probably transmitted as a sex-linked recessive [*76*]. Of great theoretical interest is the occurrence of "collagen disease," manifested by chronic arthritis or chronic dermatomyositis in a third or more of patients. Insufficient replacement therapy with gamma globulin may increase the likelihood of occurrence of the changes.

Periodic Arthralgia. Reiman and Angelides [*120*] described periodic attacks of joint pain, or at least pain in the extremities, in twenty-three members of five generations of a single family. There were no permanent residua. The attacks might start at any stage of life but once started, attacks tended to recur for the rest of a lifetime. The authors suggested that the disorder is analogous to familial angioneurotic edema. Recently, Tuqan [*172*] found amyloidosis at autopsy in a case of periodic disease and, furthermore, suggested a relationship to systemic lupus erythematosus.

Familial Primary Systemic Amyloidosis. This is a hereditary state in both mice [*65*] and men [*130*], and has been interpreted by some [*166*] as being primarily a derangement in the metabolism of connective tissue.

FINAL COMMENT

As stated near the outset, the epidemiology and genetics—the second is merely part of the first—of the rheumatic diseases are likely to become much better understood in the next few years. The interpretation of a familial aggregation in most of the states discussed in *Part II* must for the present be cautious. A pattern simulating genetic determination may result from peculiarities of the "way of life" within families. Fetal-maternal interaction is a theoretical possibility requiring investigation in connection with some diseases such as systemic lupus erythematosus. Often a conclusion of genetic influence in determining familial aggregation must be arrived at by exclusion of discoverable enviromental factors. Negative evidence is always unsatisfying. The experience with pellagra, which was once [*26*] considered by competent geneticists to be more strongly gene-determined than most would now think, has taught caution in the interpretation of diseases such as many of those reviewed here.

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Clinicopathologic Conference

Disseminated Lupus Erythematosus

STENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinicopathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Medicine, Preventive Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THIS thirty-one year old Negro woman was admitted to Barnes Hospital for the third time on April 26, 1958. She died on May 28, 1958.

The patients' first admission to Barnes Hospital was from January 25, 1952 to February 4, 1952. The patient had first been seen at the Washington University Clinics on June 22, 1951 at which time a diagnosis of intrauterine pregnancy had been made. She entered the St. Louis Maternity Hospital on January 25, 1952 and had an uneventful delivery. Her blood pressure rose during the pregnancy from 120/65 to 140/100 mm. Hg. Urine specimens examined in the clinic contained a trace to 2-plus proteinuria. A hemogram revealed a hemoglobin of 14.0 gm. per 100 ml., white blood cell count 5,050 per cu. mm. with a differential count of 62 per cent segmented forms, 3 per cent band forms, 24 per cent lymphocytes, 9 per cent monocytes, and 2 per cent eosinophils. Urinalysis on admission showed a specific gravity of 1.010, ph 6.0, 1-plus proteinuria, with many white blood cells, epithelial cells and red blood cells per high power field. The non-protein nitrogen was 14 mg. per 100 ml. During the eight days following delivery there was persistent proteinuria ranging between 1.5 to 3.2 gm. per twenty-four hours. Maximal specific gravity noted on a concentration test was 1.020. Her blood pressure on January 29 was 102/78 mm. Hg. No edema was noted; and the patient was discharged on February 4, 1952.

The patient's second admission to Barnes Hospital was from July 9, 1956 to September 21, 1956. This admission was occasioned by the development of arthritis, fever, cough, weakness and diarrhea of four weeks' duration.

The patient had been in good health until April 1956 when she experienced arthralgias in the knees, hands and elbows. Malaise, weakness and some swelling of the ankles also occurred. She was seen at the Washington University Medical Clinics where several L.E. preparations were interpreted as positive. A total serum protein was 9.2 gm. per 100 ml. with albumin 3.1 gm. per cent and globulin 6.1 gm. per cent; the reaction to the cardiolipin test for syphilis was negative. It was believed that the patient had disseminated lupus erythematosus and treatment was initiated with prednisone, 5 mg. administered four times a day.

Early in June she noted the appearance of intermittent temperature elevations of 100.4° to 103.4°F. with associated "chilly sensations," frequent watery stools and the onset of a persistent cough productive of white mucoid sputum. Shortly thereafter she began to have episodic nocturnal diaphoresis. Although myalgias and arthralgias were absent, she became progressively weaker, and lost 50 pounds during the four-week period prior to this admission. The prednisone dosage was reduced from 20 to 10 mg. per day.

About the first of July she became intermittently confused, and profoundly weak, with marked weakness of the lower extremities. Several days later her temperature rose to 40.3°C. Prednisone dosage was increased to 40 mg. per day, and the patient was admitted to Barnes Hospital.

Her past history disclosed that the patient had had cervical adenitis in April 1956 for which she had been effectively treated with penicillin and streptomycin. A tuberculin skin test at that time was negative. The systemic review was non-contributory. The patient's mother had died at the age of fifty-two years with "Lupus."

Physical examination revealed a temperature of 39.2°C., pulse 120 per minute, respirations 40 per minute and blood pressure 130/80 mm.

Hg. The patient was an acute and chronically ill obese Negro woman. The skin was hot and dry. There was no significant enlargement of the lymph nodes. The back and spine appeared normal. The pupils were equal and reacted to light and accommodation. Fundoscopic examination revealed scattered white exudates in both eyes, arteriolar narrowing and a small hemorrhage in the right eye. Although the posterior pharynx appeared normal there were scattered white plaques on the buccal mucosa. The tongue was dry. There was suggestive nuchal rigidity. The thyroid was not palpable. Fine moist rales were heard throughout the lower half of both posterior lung fields. The point of maximal cardiac impulse was in the fifth intercostal space at the mid-clavicular line. The rhythm was regular. A grade 2 apical blowing systolic murmur was detected and the pulmonic second sound was greater than the aortic second sound. The abdomen was normal. Examination of the extremities revealed a minimal effusion of the right knee, and questionable early clubbing of the fingers. Other than marked weakness and lethargy, the neurologic examination was within normal limits.

Laboratory data were as follows: hemoglobin 8.2 gm. per cent, red blood cell count 2,660,000 per cu. mm., white blood cell 4,400 per cu. mm. The differential leukocytic pattern was 1 per cent band forms, 88 per cent segmented forms, 9 per cent lymphocytes, 2 per cent monocytes. Red corpuscles showed moderate anisocytosis and poikilocytosis. Urinalysis revealed a specific gravity of 1.020, reaction 5.4, protein 2 plus, and no reducing substances or acetone. Many "rods" and "diplococci" were seen on microscopic examination of the urine sediment. Stool examination revealed no abnormalities. The reaction to the cardiolipin test was negative. Two preparations for L.E. cells were questionably positive. Two blood cultures were drawn and reported as negative. Sputum culture was not remarkable. A urine culture showed confluent growth of coliform organisms. An electrocardiogram was interpreted as showing sinus tachycardia, but was otherwise normal. Roentgenographic examination of the chest disclosed a mottled miliary nodulation throughout both lung fields and was interpreted as indicative of miliary tuberculosis. Lumbar puncture revealed an initial pressure of 290 mm. water, and a final pressure of 210 mm. water; cells with acid were 5, without acid were 4; the spinal fluid sugar was 25 mg. per 100 ml., chloride 125 mEq. per L., and protein was 57 mg. per 100 ml. Blood non-protein nitrogen was 31 mg., sugar 133 mg. and total serum proteins 6.8 gm. per 100 ml. with albumin 2.5 and globulin 4.3 gm. per 100 ml.

On July 10, 1956, acid-fast bacilli were found on sputum smear. Subsequent cultures for tubercle bacilli were also positive. Examination of the spinal fluid was repeated with essentially the same results as before and no acid-fast bacilli could be found. Bone marrow examination revealed the presence of L.E. cells. Bone marrow cultures were negative. An antituberculous regimen including the administration of streptomycin, isoniazid and para-amino salicylic acid was started. Small doses of prednisone were continued. There was essentially no change in the patient's clinical status during the succeeding eight days. She remained febrile with temperature fluctuation around 38°C. Lesions of oral moniliasis became troublesome and were effectively treated with prednisone and local gentian violet applications. Repeat lumbar puncture on July 18 revealed an initial pressure of 180 mm. water, and a final pressure of 150 mm. water. The total cell count was 58, protein was 75 mg. and sugar 32 mg. per 100 ml., chloride was 120 mEq. per L. Culture of spinal fluid for acid-fast bacilli was positive. Because of the growth of coliform organisms on culture of the urine, the patient was treated with Terramycin.® Repeat urinalysis and cultures were negative.

The patient had daily temperature elevations to 40°C. throughout the remainder of the month of July, but on August 1 her fever became less marked. During the remainder of the patient's hospitalization at Barnes Hospital there were daily temperature elevations of a more moderate degree. Because it was thought that the fever represented activity of the underlying lupus erythematosus, the prednisone dosage was increased from 20 mg. to 30 mg. per day. Her course was one of continued improvement. Several electrocardiograms taken during her hospitalization were within normal limits. Roentgenograms of the chest repeatedly demonstrated the persistence of nodular miliary mottling, interpreted as miliary tuberculosis. She was transferred from Barnes Hospital to Koch Hospital on September 21, 1956.

The patient's third admission to Barnes Hospital was from April 26, 1958 to May 28, 1958. The patient had been discharged from Koch

Hospital in October 1957, and although supposedly on maintenance dosages of isoniazid and PAS she took these medications only sporadically. In December 1957 she returned to the Maternity Clinic where she was found to be four months pregnant. At this time she noted the return of intermittent arthralgias of the hips, thighs and feet; she was again troubled by a productive cough with some hemoptysis. Physical examination was within normal limits; her blood pressure was 110/70 mm. Hg. During February she had persistent diarrhea. At subsequent visits to the clinic her weight was noted to be stable, her blood pressure remained at 110/70 mm. Hg., but 1-plus proteinuria and 1-plus glucosuria was observed during the month prior to this admission. On April 24 however, urine examination was negative for protein and reducing substances. During the month prior to admission the patient remained at moderate restriction of activity due to apparent weakness. She was admitted to St. Louis Maternity Hospital on April 26, 1958.

Laboratory data on admission were as follows: hemoglobin 9.7 gm. per 100 ml., white blood cell count 5,300 per cu. mm., leukocytic differential was 1 per cent eosinophils, 8 per cent band forms, 73 per cent segmented forms, 10 per cent lymphocytes and 8 per cent monocytes. A reaction to the cardiolipin test was negative.

On April 28 the patient delivered a viable 2,700 gm. male infant. Following delivery weakness of the lower extremities developed with arthralgias of the hips, knees and ankles. On April 29 she experienced severe abdominal cramping, diarrhea and temperature elevation to 38.4°C. She was transferred to the Medical Service on April 30, 1958.

Physical examination at that time revealed a blood pressure of 130/80 mm. Hg, pulse 100 per minute and regular, temperature 37.2°C., and respirations 20 per minute. The patient was an acutely ill, obese, Negro woman. The skin appeared normal. Examination of the fundi was unsatisfactory due to the patient's inability to cooperate, although the remainder of the examination of the eye and upper respiratory tract was within normal limits. There was no nuchal rigidity. The lungs were clear to auscultation. The left border of cardiac dullness was 12 cm. to the left of the mid-sternal line in the fifth intercostal space. A grade 2-blowing systolic murmur was heard at the aortic area. Abdominal examination was remarkable only in that the uterine fundus was palpable 3 cm. below the level of the umbilicus. No clubbing or edema was noted. Neurologic examination was of note in that there was a dull sensorium but intact orientation, marked weakness of extremities with subjective quadriceps pain on movement, hyperactive deep tendon reflexes and a positive Hoffman sign on the right. No other pathologic reflexes were noted. A hemogram on April 30 revealed a hemoglobin of 8.9 gm. per cent, white blood cells 8,950 per cu. mm., and a differential count of 8 per cent band forms, 73 per cent segmented forms, 1 per cent lymphocytes, and 8 per cent monocytes. A sickle cell preparation was negative. Urinalysis showed a reaction of 6.5, 4-plus proteinuria and 4 to 5 white blood cells per high power field. Nonprotein nitrogen was 30 mg. per cent, fasting blood sugar was 70 mg. per cent, and the erythrocyte sedimentation rate was 26 mm./hour. A roentgenogram of the chest on May 1 was interpreted as normal. At this time the patient was tachypneic and her temperature was 39.7°C. That evening a hypotensive episode developed abruptly associated with respiratory distress, gasping respirations and loud tracheal sounds. Since the cause of this episode was obscure, the patient was treated vigorously with tracheal suction, and administration of fluids, whole blood, broad spectrum antibiotics and steroids intravenously; the hypotensive status was corrected within several hours. Before transfusion the hemoglobin level was 7.5 gm. per cent, the white blood cell count was unchanged. Lumbar puncture revealed an initial pressure of 210 mm. water and a closing pressure of 200 mm. water; there were 4 cells without acid. A gynecologic consultant did not believe that the hypotensive episode was related to any pathologic condition within the pelves.

On May 2 the patient was again responsive although she remained critically ill. Blood chemical determinations on this date were: non-protein nitrogen 50 mg. per cent, blood urea nitrogen 27 mg. per cent, sodium 133 mEq. per L., potassium 4.3 mEq. per L., carbon dioxide 13.1 mEq. per L., chloride 132 mEq. per L. L.E. preparations were positive on two occasions. Total serum proteins were 6.9 gm. per cent and the globulin was 4.1 gm. per cent. The hemoglobin was now 13.9 gm. per cent, and the white blood cell count was 12,400 per cu. mm. It was noted that the patient

was oliguric. The oliguria persisted for the succeeding five days during which time she was maintained on appropriate fluid replacement therapy, with the maintenance of satisfactory electrolyte balance. The non-protein nitrogen however, rose to 138 mg. per cent. On May 5 diuresis occurred and the elevated non-protein nitrogen slowly receded. A low pitched grade 3 systolic murmur at the fourth intercostal space in the left mid-clavicular line along and a grade 1 early blowing diastolic murmur at the third intercostal space along the left sternal border were first detected at this time. An electrocardiogram revealed T wave flattening and inversion in almost all leads and was interpreted as showing anterior myocardial ischemia. Electrocardiograms on the following two days remained unchanged and were believed to be compatible with myocarditis. Multiple blood cultures obtained during this period and throughout the remainder of the patient's life were negative.

The patient's condition remained critical during the next five days but there were no changes in her cardiovascular status. She was seen again by a gynecologic consultant because of failure of the episiotomy wound to heal. Appropriate local measures were instituted along with reduction in the dosage of steroids. A culture of the episiotomy wound was reported as showing "heavy growth of mucoid lactose fermenters." On May 13 the hemoglobin was 11.0 gm. per cent, white blood cells 9,250 per cu. mm. and the differential count revealed 83 per cent segmented forms, 16 per cent lymphocytes, and 1 per cent monocytes.

An infection of the urinary tract was suspected because of the previous use of an indwelling catheter, and therapy with chloramphenicol was instituted.

On May 21, a gallop rhythm was heard, with a cardiac rate of 140 per minute. The next day a chest roentgenogram was interpreted as showing interval development of considerable generalized cardiomegaly but without significant pulmonary congestion, and the patient was digitalized rapidly. An electrocardiogram on May 25 revealed atrial flutter with incomplete auriculoventricular dissociation and rapid nodal rhythm. Because of the appearance of intermittent runs of ventricular tachycardia, considered to be related to digitalis intoxication, she was given Pronestyl.® Two days later, her rhythm reverted to normal sinus rhythm but she remained febrile with the same murmurs and gallop sounds. Her hemoglobin was 9.2 gm. per cent, and she remained in a critical condition. On May 27 an electrocardiogram revealed a persistence of the lateral myocardial ischemia with a normal sinus rhythm. On May 28 she died suddenly at 3:00 A.M.

CLINICAL DISCUSSION

DR. SOL SHERRY: The patient under discussion was suffering from systemic lupus erythematous. Although the protocol gives the impression that her disease had its onset in 1956, there is one note in the chart which suggests that it was more chronic. According to this note the patient had been visiting the clinic for many years; a diagnosis of Osgood-Schlatter disease had been made at the age of eleven because of the presence of knee pains; and the patient had had persistent difficulty with arthralgias, especially of her knees, up to the time of her first pregnancy. After the first pregnancy in 1952 there was an aggravation of muscle and joint pain which persisted until the recorded exacerbation in 1956. The note also states that the exacerbation in April 1956 was immediately preceded by the appearance of cervical adenitis. Penicillin was given and was followed shortly by itching of the skin and a questionable eruption. Shortly thereafter the symptoms appeared which led to the diagnosis of systemic lupus. The history records that the patient's mother died of lupus. Was this ever corroborated?

DR. THOMAS BRITTINGHAM: I went to see the prosector who performed the postmortem on her mother. Systemic lupus was the final diagnosis.

DR. SHERRY: Do you know of any familial basis for lupus?

DR. BRITTINGHAM: Yes. Harvey reports that more than one member of a family may be affected with lupus. In addition Shearn noted systemic lupus in two members of the same family. Davis reported its occurrence in identical twin sisters.

DR. SHERRY: Dr. Eisen, in recent years several authors have referred to lupus as an autoimmune disease. Are you in agreement with this concept?

DR. HERMAN EISEN: I would like to consider first whether or not there are truly autoimmune phenomena present in lupus. If there are, I should then like to consider whether or not they are responsible for a major part of the morbidity

in this disease. Practically everyone would agree that an autoimmune phenomenon differs from a classic immune one, only in respect to the fact that the antigenic determinants are intrinsic constituents of cells or extracellular substances in the body. If this is the case, then one would require of a presumptive autoimmune phenomenon that it is inducible and specific. Many reactions which occur in lupus with a high frequency, such as the positive L.E. test, hemolytic anemia, thrombocytopenic purpura and white cell and platelet agglutinins, certainly appear very suggestive as regards their being autoimmune. With respect to the positive reaction to the cardiolipin test, the evidence is strong that this represents a specific interaction between a constituent of normal body cells, cardiolipin and a globulin, and that complement is fixed in the process. In this connection may be mentioned recent work from several laboratories on the lupus cell factor itself, which now appears clearly to be a gamma globulin with all the physical attributes of antibody, and which interacts with nuclei and isolated nuclear constituents, and binds complement in the process. In some cases this gamma globulin even gives precipitin reactions with nuclear constituents. These reactions are specific in the sense that only a very small fraction of the total globulin mass is capable of interacting with the particular cellular constituents mentioned. Whether or not they are truly immune, however, depends on whether or not the formation of these specific globulins is induced by the cellular constituents with which they subsequently interact. As far as I know, this has not been demonstrated, at least in lupus, and therefore I think one has to be cautious about inferring that these reactions are truly autoimmune. Do these reactions cause morbidity? I think some of them probably do, e.g., in the case of the positive Coombs' reactivity, platelet agglutinins and perhaps the white cell agglutinins. In the case of cardiolipin reactivity, I know of no evidence which suggests that cardiolipin-anticardiolipin interaction will produce morbidity. In the case of the L.E. factor, I have reservations regarding its role in morbidity because there is a good deal of evidence which says that antibodies will not penetrate through normal cell membranes to react with intracellular constituents. For example, intracellular parasites and some intracellular enzymes seem to be completely protected from the action of extracellular homologous antibodies. From studies that we have recently performed, it appears also that proteins do not penetrate into isolated lymph node cells. Of course, the L.E. factor could interact with nuclear material liberated by cell disruption, and such an interaction could then initiate allergic inflammation just like many other antigen-antibody interactions do. One final point: if the phenomena we are talking about turn out eventually actually to be autoimmune, account will have to be taken of some more primary events which make possible the development of antigenic determinants from what are otherwise normal constituents of the body. In other words, for truly autoimmune systems to develop spontaneously, a primary process would have to occur which converts normal body constituents into antigenic determinants. Such a primary process would, I imagine, come pretty close to being the prime mover in any autoimmune disease.

Dr. C. V. Moore: Certain workers now believe that the lupus factor reacts, not only with nuclear constituents, but specifically with the DNA of the nucleus in lupus. I wonder if Dr. Eisen would comment on the specificity of the reaction.

Dr. Eisen: The only work that I am familiar with in this regard is that of Robbins, Holman, Deicher and Kunkel, who showed that there occurs a specific interaction between lupus serums and purified DNA obtained from widely different sources: pneumococcal DNA, calf thymus DNA and salmon sperm DNA. These all interact with lupus serums with fixation of complement, and in some cases with the formation of precipitates. There is no species specificity here, but there seems to be specificity for DNA. If the reaction is really with DNA, and not with trace amounts of contaminating protein, it is particularly interesting because for a long time many have looked for, but failed to find, substantial evidence that DNA can act as an antigenic determinant.

Dr. Sherry: Is it your impression that the serum factor responsible for the lupus phenomenon is pathogenetically involved in the development of this disease or is it simply an associated phenomenon seen in lupus?

Dr. Eisen: At present I am inclined to guess that the serum factor involved in the lupus phenomenon is an accompaniment of the disease, rather than a basic cause of morbidity in this disease. I believe this because the evidence is strong that antibodies do not penetrate into

normal cells. If they do not penetrate, they can not interact with nuclei to make normal cells sick. This simple view is very likely too simple, but I would want some more evidence before becoming concerned with more complicated possibilities.

DR. SHERRY: Dr. Kipnis, we recognize that partially depolymerized DNA is present in the phagocyted material of the lupus cell, and in the hematoxylin found in the tissues of patients with this disease. However, in addition, there is a considerable amount of fibrinoid present in the connective tissues. What are the present concepts concerning the nature of fibrinoid?

DR. DAVID KIPNIS: The term fibrinoid was introduced by Neumann in 1880 to describe an intensely eosinophilic, homogeneous, dense, refractile substance with staining characteristics similar to those of fibrin. This material is found in a great variety of conditions in man ranging from degenerative lesions in the placenta and inflammatory lesions of the appendix to the afflictions listed under the generic terms collagen, hypersensitivity or connective tissue diseases. There has been considerable controversy as to the origin of fibrinoid material. The availability of improved histochemical and histoenzymatic methods and the recent introduction of specific antibody tagging technics have made feasible a partial clarification of this problem. The concept that fibrinoid is derived from degenerating collagen is no longer tenable in view of the fact that the chemical composition of fibrinoid differs from that of collagen, and its staining characteristics are not altered by collagenase. Furthermore, fibrinoid does not appear to be formed by an alteration of the ground substance of connective tissue since fibroinoid and ground substance also differ as to their chemical composition and their response to treatment with various enzymes such as hyaluronidase (which hydrolyzes ground substance) and plasmin (which hydrolyzes fibrin and fibrinoid). It appears most likely from a survey of the available evidence that fibrinoid derives from plasma proteins which have extravasated into the connective tissues presumably through damaged vascular endothelium. Several groups of investigators using the fluorescent antibody tagging technic have demonstrated that of the plasma proteins, only fibrinogen or some derived polymerized form, such as fibrin, is present in all lesions associated with an accumulation of fibrinoid material. These lesions may differ in appearance in varying conditions depending on the degree of admixture of fibrin and other plasma proteins with collagen, elastic and reticulin fibers, ground substance and the cellular elements of connective tissue. At the present time, I am unaware of any histochemical or histoenzymatic evidence demonstrating a primary disorder in the metabolism of connective tissue in lupus erythematosus.

DR. SHERRY: After the diagnosis of systemic lupus was established on the basis of clinical and laboratory evidence in April 1956, steroid therapy was instituted and then maintained. Three months later, a febrile illness developed with productive cough, night sweats, weakness and mental confusion. The patient was admitted to the medical service for the first time, where a diagnosis of miliary tuberculosis, tuberculous meningitis and buccal moniliasis was made. What were the roentgenographic findings on that first hospital admission?

DR. HARVEY A. HUMPHREY: The initial clinic film revealed the lungs and heart to be perfectly normal. At the time the patient was admitted to Barnes Hospital, miliary infiltrates were visualized throughout the lungs bilaterally, symmetrically, and in a uniform distribution. The diagnosis was miliary tuberculosis. The heart appeared normal at that time. During the ensuing five weeks there was a definite decrease in the appearance of the miliary infiltrates. We were not able to uncover a primary focus anywhere in the lungs. During the next eight days there were three or four examinations of the chest. The patient apparently complained of pain in the right side of the chest, and a pulmonary infarct was suspected clinically. This was also suspected at the time the roentgenogram was studied, because of the appearance of a zone of confluent infiltration abutting on the pleura at the right base. By the time the patient was discharged, there was only a slight, localized, persistent increase in density. The miliary disseminated disease in the lungs however appeared to be slightly increased.

DR. SHERRY: Dr. Goldman, would you comment on this patient's tuberculosis? The reaction to the tuberculin test was negative when she was first seen in the clinic in April 1956, and yet three months later she had miliary tuberculosis and tuberculous meningitis.

DR. ALFRED GOLDMAN: I would like to know

what type of tuberculin test was performed, that is, whether it was a first strength or intermediate.

Dr. Brittingham: It was intermediate strength.

Dr. Goldman: There are now many cases on record in which miliary tuberculosis and meningitis occurred in patients with lupus who were receiving steroid therapy. We have every reason to believe that the steroids were a factor in the pathogenesis of her tuberculosis, at least in the aggravation of a latent tuberculosis.

Dr. Sherry: Does tuberculosis activated by steroid therapy produce any problems in therapy?

Dr. Goldman: No. The tuberculosis which develops is either the miliary or exudative type, both of which respond best to antibiotic therapy. The steroids apparently interfere with fibrosis, with fixation of the lesions, with clotting of vessels around the lesion and the organisms then get out into the circulation.

Dr. Sherry: Do you believe this patient had adequate therapy for her tuberculosis?

Dr. Goldman: She apparently did very well. There is no evidence that she had tuberculosis, at least in the chest, when she came back after her stay at the Koch Hospital. However, we would have continued her chemotherapy for a period of at least three years. Apparently, she more or less stopped therapy after about thirteen months.

Dr. Sherry: Do you believe that the tuberculosis played any further role in her subsequent illness?

Dr. Goldman: I certainly would be surprised for we do not have any evidence that she had a recurrence of her tuberculosis.

Dr. M. Kenton King: Will steroid therapy reduce the spinal fluid cell count?

Dr. Goldman: Yes, it does in tuberculous meningitis. Actually there are many people today who will treat meningitis with steroids for just that reason.

Dr. Sherry: From time to time, at these conferences, we are impressed with the sudden development of severe overwhelming infections occurring in patients maintained on long term steroid therapy. Dr. Magee, would you comment about your experiences with this problem?

Dr. W. E. Magee: We found that 30 per cent of patients with secondary fungal infections had been treated with cortisone or some form of steroid therapy. It was our impression that perhaps steroids were implicated. It should be noted that these patients had severe primary disease, such as leukemia or lymphoma, and one had lupus.

Dr. Sherry: Dr. Harford, what is the present status of steroids as they relate to immunity?

Dr. Carl Harford: The main information concerning the mechanisms by which steroid therapy lowers resistance to infection has been obtained by controlled experiments in animals, but it is probably applicable also to human beings. Under these circumstances, the doses of steroid which have been employed have usually been very large. In experimental bacterial infections in which the animals have been treated with steroids, one observer noted decreased numbers of phagocytic cells and increased numbers of bacteria which seemed to be growing in edema fluid. Inflammation, in general, appears to be decreased, as judged from a number of various experiments. One of the most interesting of these is the rabbit ear chamber technic. A window is made in a rabbit's ear and then connective tissue grows in through the window and can be observed under the microscope. Under these circumstances, it is possible to produce either infection or other kinds of injury to the tissue, and to show that cortisone diminishes the number of phagocytic cells which get into the injured or infected focus. Characteristically in such inflammation the terminal capillaries become dilated and, at least some of the time, steroid therapy causes a reconstriction of these blood vessels. This reconstriction is considered to be a possible mechanism by which the steroid may interfere with the migration of phagocytic cells. There are also experiments which indicate that steroids affect capillary tone as directly visualized in the blood vessels of the rat meso-appendix. On the other hand, steroids apparently do not have any effect on phagocytic cells as studied *in vitro*. Motility is not decreased and actual ability to ingest organisms is not decreased. I have no information reported on the effect of steroids on the ability of a phagocyte to destroy an infectious agent after ingestion. It should also be pointed out that steroids have no effect on growth of bacteria *in vitro*, insofar as they have been tested. Now, as far as antibodies are concerned, passively immunized animals have no change in the antibody level with steroid therapy. However, it has been possible to show that with large doses

of steroid, levels of antibody can be reduced in actively immunized animals. On the other hand, there is always some antibody, so that it seems unlikely that interference with antibody formation is an important factor in reducing resistance of animals to infection. As far as toxins are concerned, there is no apparent effect of steroid therapy on exotoxins, such as diptheria toxin, but there are very striking effects on endotoxins. Whether steroids protect against endotoxins, or potentiate their action, seems to depend on the details of just how the experiment is done. In summary, the major effect of steroids in infection appears to be an inhibition of the migration of phagocytes, thereby an interference with phagocytosis. However, I suspect that the problem is much more complicated than it appears to be at the present time. To give an example: In poliomyelitis of the hamster, when the animals are not treated with steroid, the disease is mild with occasional paralysis. On the other hand, in animals treated with steroid, the disease becomes an overwhelming, fulminant one, in which the animals die. It is difficult to account for this type of experiment on any theories concerning phagocytosis, because as far as I can tell there is no relationship of phagocytosis to the pathogenesis of this viral infection.

DR. SHERRY: Although our patient responded to antituberculous and local antifungal medication, her course was complicated by an infection of the urinary tract and an exacerbation of the activity of her systemic lupus. Eventually she improved sufficiently to be transferred to Koch Hospital. After a year at that institution she was discharged, immediately became pregnant and soon had a recurrence of her athralgias. She carried to term, entered the hospital in April 1958 and delivered a viable male infant. Immediately after delivery her lupus seemed to flare again and she was transferred to the medical service for her second and final admission. Since lupus is most commonly a disease of women in the child-bearing age, I wonder, Dr. Woolf, whether pregnancy, in your experience, has a predictable effect on the course of lupus?

Dr. RALPH WOOLF: From review articles involving the study of approximately 100 patients, pregnancy does not have a predictable effect on the course of lupus except for a transient worsening immediately after the termination of pregnancy. In the acute disseminated variety of lupus, 45 per cent of the mothers became worse or died; 55 per cent of the mothers were unaffected or better.

DR. SHERRY: Would this hold true if nephritis were also present?

DR. WOOLF: In that particular instance you would anticipate that there would be more renal disease. In other words, pregnancy superimposed on a basic renal lesion would undoubtedly aggravate the renal disease.

DR. SHERRY: The three major areas of interest in this patient's final hospitalization relate to her renal lesions, cardiac disease and the possibility of some other complication contributing to her death. Dr. Bricker, what do you believe will be found in this patient's kidneys at postmortem examination?

DR. NEAL BRICKER: There are three different types of renal disease that must be considered. First the evidence is good that she sustained acute tubular necrosis, and that she subsequently recovered from this. Her untimely death occurred at a point soon enough after the acute tubular necrosis so that we might very well expect to find residual evidence in the tubular epithelial cells and in the basement membranes of the tubules. Second, the possibility of pyelonephritis must be seriously considered. The patient had an inlying catheter on two different occasions, and she had bacteriuria on both of these occasions. During the second episode, the bacteriuria was severe. We know that this would make her a candidate for pyelonephritis and the chances of her having this lesion are probably greater than 50 per cent. The third and final category of lesions is, of course, that associated with systemic lupus erythematosus. Clinically, there may be no manifestations of renal disease or conversely there may be varying degrees of renal insufficiency, including a picture of terminal uremia. Histologically, a similar spectrum exists. In at least 70 per cent of patients with systemic lupus, the basement membrane lesion is found in the glomeruli. In more advanced forms of lupus nephritis, the kidney may be histologically identical with chronic glomerulonephritis. There is no absolute evidence that this patient had lupus nephritis and indeed her renal function was well preserved until the occurrence of acute tubular necrosis. However, because of the presence of proteinuria on several different urinalyses, covering a period of several years, and on purely statistical grounds, I would think that there will be glomerular lesions consistent with lupus.

DR. SHERRY: You believe the kidneys will demonstrate three types of lesion: lupus nephropathy, pyelonephritis and evidence of regenerating tubular epithelium? How severe do you believe pyelonephritis will be?

DR. BRICKER: We are a little handicapped in making this decision. We do know that her renal function returned to near normal levels after recovery from acute renal failure, and hence we cannot postulate that she lost a major portion of her nephron population. On the other hand, the setting is right for a wide-spread pyelonephritis, and we must hold open the possibility that she could have either small areas of scattered focal pyelonephritis, or severe diffuse, acute chronic pyelonephritis.

DR. SHERRY: During the final part of this patient's illness there was considerable interest in the nature of her cardiac involvement. During the diuretic phase of the acute tubular necrosis, a systolic murmur developed in the fourth intercostal space at the mid-clavicular line, and a diastolic murmur along the left sternal border, as well as electrocardiographic changes compatible with myocarditis. Subsequently, there was the appearance of gallop rhythm, tachycardia, transient arrhythmias and an enlarging heart. Dr. Humphrey, would you discuss the chest roentgenogram taken during the patient's last admission?

DR. HUMPHREY: The supine film taken on admission showed an essentially normal heart. The last film was obtained about one week before the patient died. It showed a very striking increase in the heart size. There was no obvious pulmonary congestion. The question, naturally was raised as to whether or not this was a sudden increase in heart size due to accumulation of pericardial effusion, or due to acute dilatation. The absence of congestion would be in favor of a pericardial effusion. However, the shape of the heart had not changed appreciably and was more suggestive of an acute dilatation, presumably on the basis of a carditis.

DR. SHERRY: Dr. Massie, you had the opportunity to review the electrocardiograms. What would your interpretation be as to the nature of her cardiac disease?

DR. EDWARD MASSIE: We have a number of records, which were normal until May 5, 1958. On this day the T waves became somewhat inverted in the V leads. This change could be read as ischemia or myocarditis and/or pericarditis. In view of the suspicion of L.E., the latter diagnosis seems most likely. Subsequent records showed this finding as well as arrhythmias. Arrhythmias are not too common in lupus with cardiac involvement, but here we have another explanation, namely digitalis. It is noteworthy that as the administration of digitalis was discontinued, the record changed from A-V dissociation with nodal rhythm to flutter to fibrillation and terminally the patient had a normal sinus rhythm with a fairly good looking tracing. With regard to the heart itself, I think it fair to say that this patient's symptoms fit into the syndrome of Libman-Sacks disease. She should have verrucous endocarditis and if I had to choose the valve involved, it would be the aortic valve. The murmurs seemed to be more associated with the aortic valve than any other valve, although we do know there was a systolic murmur at the apex. It is also important that this patient was anemic, had fever and was quite sick, all conditions which in themselves may produce murmurs. Forty per cent and in some series even 50 to 60 per cent of patients with disseminated lupus have atypical verrucous endocarditis, so this would certainly be a most likely finding here.

DR. SHERRY: Does the presence of verrucous endocarditis cause heart failure?

DR. MASSIE: No, actually the murmurs may be present in such patients without any endocardial lesion. I do not think the endocarditis alone will produce heart failure. These patients have myocarditis and pericarditis and I suspect the combination plus anemia and infection contribute to the death of the patient.

DR. SHERRY: But Libman-Sacks disease refers to the atypical verrucous endocarditis alone?

DR. MASSIE: Yes, just that.

DR. SHERRY: But you would say that this patient had more than Libman-Sacks disease; that she probably had myocarditis and pericarditis as well?

DR. MASSIE: Yes, I would certainly include those lesions in this particular patient.

DR. SHERRY: Do the Libman-Sacks vegetations ever embolize?

DR. MASSIE: Yes, and as a matter of fact, I would not be surprised to see pulmonary emboli here.

DR. SHERRY: It was my impression that Libman-Sacks vegetations did not embolize, and that when embolic phenomenon occurred one should suspect either a superimposed bacterial endocarditis or a non-bacterial thrombotic

endocarditis. Do you believe that this patient had a superimposed bacterial endocarditis or a non-bacterial thrombotic endocarditis?

DR. MASSIE: I do not think so.

DR. SHERRY: Dr. Smith, do you agree with Dr. Massie, or are there other ideas which you would like to express?

DR. JOHN SMITH: I agree with Dr. Massie. This patient probably had Libman-Sacks endocarditis and the murmurs can be explained on that basis. The endocardial verrucous lesions that form in this disease are apt to form on the mural endocardium under the valves, or at about the margins of their attachments. These lesions interfere with the motion of the valve and produce little or no destruction or distortion of the valvular elements themselves. They are flat-based and rather tenacious and tend only infrequently to break off and embolize. The cause of congestive failure in these patients has been a matter of mystery. In patients with lupus the myocarditis which develops is apt to be random and focal. It would seem that the lesions are not sufficiently numerous to cause extraordinary anatomical disruption of cellular elements and to break down myocardial function from that standpoint. Therefore it seems more likely that the congestive failure in these patients is a matter of physiological exhaustion of the muscle, whatever that may imply. I believe in addition that pulmonary emboli may well have occurred. There seems to have been an infarct in the past (on her second admission) and this, of course, is a surreptitious disease. It can occur in any debilitated stage and in the terminal state of any disease. The lungs may be fairly generously seated with emboli, but it would not surprise me to find no such lesion either.

DR. LILLIAN RECANT: In relation to the question of pulmonary emboli, we have not yet mentioned the precipitating factor for the acute tubular necrosis. As I recall, this was described as an episode in which the patient became suddenly tachypneic and then hypotension developed. Might this not have been a pulmonary embolus?

DR. SMITH: Yes, the story is quite typical of one and I visualize that it very likely was.

DR. SHERRY: I agree. Pulmonary embolization may be underlying this whole problem. Since the patient was receiving steroid and antibiotic therapy, and since she had a debilitating disease, we may suspect the possibility of an underlying disseminated infection as well.

DR. HUGH CHAPLIN: Did the patient receive penicillin during her final hospitalization?

DR. SHERRY: Yes.

DR. CHAPLIN: The reason I ask is that false-positive L.E. tests have been reported in patients having sensitivity reactions to penicillin. It sounds as though she may have had a penicillin sensitivity reaction when she was treated for cervical adenitis in April 1956. I wonder if Dr. Eisen would comment on the validity of positive L.E. tests under conditions of suspected penicillin sensitivity.

DR. EISEN: When a positive L.E. cell test is found in someone having an acute or subacute penicillin reaction, it is useful to suspect underlying lupus. It would be nice to know just what the incidence of positive L.E. cell preparations is in individuals with clinically well established penicillin reactions. I suspect it would be extremely low, and would be surprised if a strongly positive L.E. cell test was obtained purely as a consequence of an allergic reaction to penicillin.

DR. SHERRY: We may offer as our final diagnosis systemic lupus erythematosus with involvement of various organs including the heart and kidney; acute and chronic pyelonephritis; healing acute tubular necrosis; probably inactive tuberculosis; possible disseminated infection; and possible pulmonary embolization. Dr. Meyer will present the gross findings.

PATHOLOGIC DISCUSSION

DR. JOHN S. MEYER: At autopsy we saw an obese young woman with pretibial pitting edema. The right pleural cavity contained 100 ml. of cloudy fluid. Fibrous adhesions were present over the lower right lobe and numerous small (1 to 2 mm.) fibrocaseous nodules were in this lobe. Similar nodules were found in the spleen, liver and kidney, and the hilar lymph nodes were enlarged (2 to 3 cm.) and caseous.

The uterus was enlarged and the endometrium thick, spongy and covered with creamy pus from which we cultured Proteus mirabilis and an unidentified species of streptococcus (similar organisms were cultured from the urinary bladder).

The heart was moderately hypertrophied (450 gm.) and dilated and a few petechiae were present in the epicardium. When the heart was opened two types of lesion were observed. On the atrial surface of the posterior leaflet of the mitral valve near the closing edge was a firmly ad-

FIG. 1. The "massive thrombotic" type of verrucae sometimes seen in patients with lupus erythematosus. The more common type is a small, flat lesion beneath the mitral or tricuspid valve.

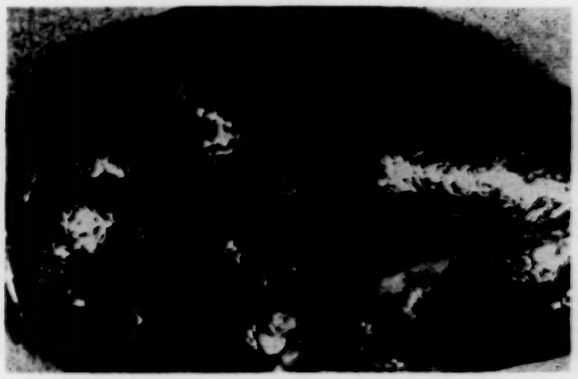

FIG. 2. A finely granular kidney with multiple petechiae.

herent, sessile, tawny, granular mass (2 to 3 mm. across and less than 1 mm. thick). On the ventricular surface of the aortic valve cusps (Fig. 1) and covering one of the commissures were three large granular, reddish yellow, friable masses measuring up to 6 mm. in diameter and length. A similar mass was present in the right ventricular cavity 2 mm. below the tricuspid valve. These masses on the aortic valve and beneath the tricuspid valves were only loosely attached with no ulceration of the underlying tissue. The coronary arteries appeared normal but several yellow, necrotic appearing areas were seen in the myocardium of the left ventricle. Multiple infarcts were found in the brain, spleen and kidneys.

The kidneys weighed 350 gm. together, were finely granular and had multiple petechiae on their surfaces. (Fig. 2.) In addition, several large, dark red, more coarsely granular depressed areas were seen. The cortices were yellow, somewhat thin throughout (5 to 6 mm.) and contained a few petechiae. The mucosa of the bladder was hemorrhagic. The liver was slightly enlarged (1,800 gm.) and showed intense central congestion.

DR. WILBUR A. THOMAS: It is evident that we have several conditions to consider. The only important site of pyogenic inflammation was in the uterus with lesser amounts in the urinary system. On microscopic examination we saw a moderate degree of chronic pyelonephritis and one focus of acute pyelonephritis but other features were more prominent.

The only residua of the miliary tuberculosis were small nodules in the lower lobe of the right lung, spleen, liver, kidney and lymph nodes. Microscopically, these consisted largely of fibrous tissue with only minimal caseation. No tubercle bacilli were demonstrated by stains or special cultures.

Most of the remaining lesions can be attributed directly or indirectly to lupus erythematosus. The endocardial masses consisted largely of fibrin partially covered with endothelial cells and contained no bacteria. The reaction in the valve beneath was minimal consisting principally of endothelial proliferation and mononuclear cell infiltration. Hematoxylin bodies were not found here or elsewhere. The appearance of the flat verruca on the mitral valve was that of the common variety of Libman-Sacks' endocarditis but it would have been more characteristic if it had been found in the pocket beneath the valve. The lesion associated with the tricuspid valve was in its pocket, but it and the masses on the aortic valve were considerably larger and more friable than the usual Libman-Sacks' process. However, this appearance of verruccae in lupus erythematosus has been seen occasionally before. It was described by Gross [*1*] twenty years ago as one of three types of endocarditis that may occur in the disease. Gross' name for it was the "massive thrombotic" type and for the other two the "flat spreading" and the "pyramidal ridge" types. The appearance of the "massive thrombotic" type is quite similar to that of non-bacterial thrombotic endocarditis and only the gross distribution of the lesions and the association with other more characteristic stigmas of lupus erythematosus enable us to make the distinction. It is the only type of the three that is likely to give rise to thromboemboli. The multiple infarcts that were found in the heart, brain, kidney and other organs were probably due to embolization. Arteritis was not noted anywhere, and the

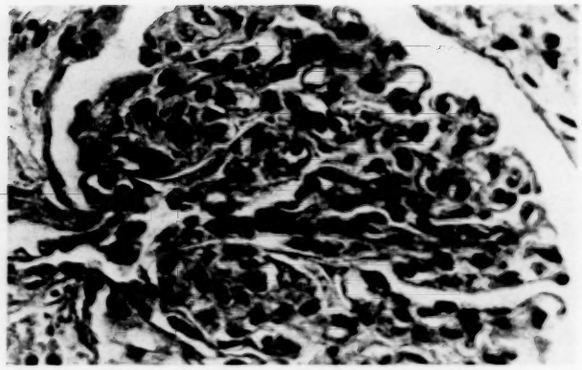

FIG. 3. A glomerulus with thickened basement membranes including the "wire loop" lesion characteristic of lupus erythematosus. Hematoxylin and eosin × 800.

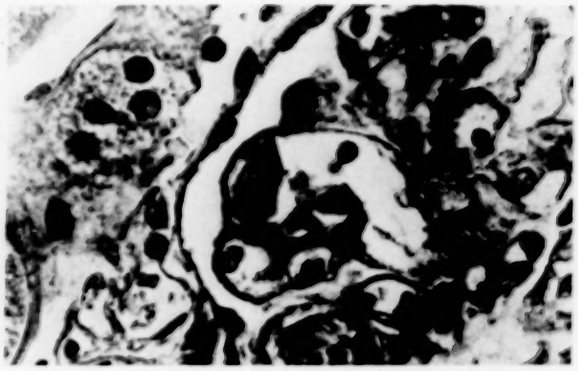

FIG. 4. Higher magnification of another glomerulus with thickened basement membranes and also an adherent mass of "fibrinoid" material within a capillary. Hematoxylin and eosin × 1800.

splenic arteries did not have an "onion peel" appearance.

We have already mentioned miliary tubercles, infarcts and pyelonephritis in the kidneys. However, all three of these changes appeared to be relatively minor compared with glomerular changes that are characteristic of lupus erythematosus. (Figs. 3 and 4.) Moderate to marked thickening of the basement membranes was noted in almost every glomerulus. In many this change was especially prominent in peripheral loops giving the typical "bent wire" or "wire loop" appearance. Some glomeruli showed fibrinoid change of a portion of the tuft.

Our knowledge of the glomerular changes in lupus erythematosus has been increased recently by application of the technics of electron microscopy to renal biopsies [*2*]. The glomerular changes, at least in the early stages, are largely in the basement membranes and endothelial cells with relative sparing of the epithelial cells (in contrast to subacute glomerulonephritis). The thickening is due partly to actual increase in size of the basement membrane and partly to amorphous material (? fibrinoid origin) pressed against it.

The final anatomical diagnoses are disseminated lupus erythematosus with "wire-loop" thickening of basement membranes of glomeruli and necrosis of glomeruli, and verrucous thrombi on ventricular cusps of aortic valve (large), on atrial surface of mitral valve (small) and on ventricular surface of tricuspid valve (small); organizing thrombus in right atrial appendage; thrombi and multiple small infarcts in spleen; thrombi and multiple small infarcts in kidneys; organizing thrombus in left tibial vein; multiple thrombi in small cerebral arteries with infarcts in left corpus striatum, right thalmus and hypothalmus and left side of pons; thrombi in small coronary vessels of left ventricle with focal infarcts; hypertrophy and dilatation of heart, slight (450 gm.); atrophy of adrenal cortices (history of prednisone therapy); congestion of lungs and liver with central necrosis in liver; slight increase in fluid in pleural and pericardial spaces (25 ml. right pleura, 100 ml. left pleura and 15 ml. in pericardial sac); pyelonephritis, chronic and focal acute; hypertrophy of uterus with hemorrhages into cervix; organizing thrombi in myometrial arteries and veins; purrulent endometritis and myometritis; small fibrocaseous nodules in lower lobe of right lung, hilar and subpleural lymph nodes, spleen and kidneys (history of dx of miliary tuberculosis one year prior to death arrested by chemotherapy; no acid-fast bacilli demonstrated by culture or stain at autopsy).

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Case Reports

Bilateral Adrenalectomy for Hypertensive Vascular Disease*

Hormonal Requirements in the Presence of Renal Insufficiency: Body Potassium in Chronic Hyperkalemia

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PRELIMINARY observations on the effects of bilateral total adrenalectomy for hypertensive vascular disease have been reported from this laboratory [*1–3*]. One of the patients, whose early postoperative course was reviewed in these previous reports, and also by Moore and Ball [*4*], survived for four and a half years after removal of all adrenal tissue, and it is the purpose of this paper to report some observations made during this period and to review the findings at autopsy. Three points in particular are noteworthy: (1) There was long-sustained relief of cardiac failure postoperatively despite a very slight reduction in blood pressure level. (2) Renal insufficiency developed slowly in these four and a half years, and as the insufficiency progressed there was an increasing requirement for both salt and salt-retaining hormone. (3) Despite the presence of chronic hyperkalemia with intermittent clinical episodes of acute potassium intoxication, the total exchangeable body potassium was found to be within normal limits.

CASE REPORT

A thirty-four year old machinist was admitted to the Peter Bent Brigham Hospital on July 6, 1950, complaining of shortness of breath, swelling of the ankles and distention of the abdomen. In 1940 he had been rejected by the Armed Forces because of "high blood pressure," but he was free of symptoms at that time. He had experienced severe headaches for several months in 1944 and was found to have proteinuria at that time. Elevated blood pressure levels were recorded repeatedly but he remained well until a month prior to his first admission here, when he first noted shortness of breath on exertion, abdominal distention with cramps and diarrhea, and paroxysms of dyspnea at night. Nine days before admission acute pulmonary edema and fever had developed.

His health in the past had been good. For many years he had been troubled by seasonal hay fever, sometimes associated with wheezing respirations. The patient's mother was reported to be hypertensive, and his father died at the age of sixty with "heart failure secondary to asthma."

Examination on July 6 revealed the patient to be a well developed, well nourished white male with slight cyanosis and apparent respiratory distress. The temperature was 100°F., pulse 110, respirations 24, blood pressure 150/120 mm. Hg. There was spasm and tortuosity of the retinal arterioles, but no hemorrhage, exudate or papilledema was seen. Inspiratory and expiratory sibilant rhonchi and moist rales were heard over both lung bases posteriorly. The heart was enlarged, with the apex beat displaced to the left anterior axillary line in the fifth interspace. The rhythm was regular, but a protodiastolic gallop was present. No murmurs were heard. The abdomen was somewhat distended, and the liver edge was palpated 4 fingerbreadths below the right costal margin. There was slight sacral and ankle edema.

* From the Departments of Medicine, Surgery and Pathology, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Massachusetts. Supported in part by grants from the John A. Hartford Foundation, Inc., New York City; the Eugene Higgins Trust Fund of the Harvard Medical School; the United States Public Health Service, Bethesda, Maryland; and Ciba Pharmaceutical Products Inc., Summit, New Jersey.

Urinalyses revealed 2 to 4 plus proteinuria. The sediment contained numerous hyaline and granular casts and up to 5 leukocytes per high power field. The blood Hinton test was negative. The hematocrit was 44 per cent and the leukocyte count was 8,000 per cu. mm. with a normal differential count. The fasting blood sugar, serum sodium, chloride, potassium and bicarbonate concentrations were normal. The blood urea nitrogen was 29 mg. per cent. The total serum protein concentration varied from 4.4 to 6.0 gm. per cent with an albumin concentration of 2.8 and 3.2 gm. per cent on two occasions. The urine specific gravity following a brief period of fluid restriction was 1.010 (corrected for proteinuria). The phenolsulphonphthalein excretion was 55 per cent in 135 minutes, and the glomerular filtration rate was 32.5 ml. per minute (19.5 ml. per minute per sq. M). The venous pressure was 250 mm. saline solution, the arm-to-tongue circulation time was forty-five seconds (Decholin®) and the vital capacity 2.4 L. The electrocardiogram was characteristic of left ventricular hypertrophy. Fluoroscopy of the heart demonstrated marked enlargement with a generalized increase in all diameters. A standard chest x-ray film showed evidence of pulmonary vascular congestion with an area of pulmonary edema below the left hilum. The transverse cardiac diameter was 183 mm. (predicted 129). Retrograde pyelography showed the kidneys to be reduced in size. A total body water determination on July 25 was 36.2 L. or 59.3 per cent of body weight (deuterium oxide dilution method) [*5,6*].

A program of treatment for congestive failure was undertaken, with administration of digitalis, restriction of dietary sodium and administration of Mercuhydrin® intermittently. The patient's dyspnea decreased and he lost 8 kg. in weight. The vital capacity increased to 3.4 L. However, it was apparent that the patient had severe hypertensive vascular disease with cardiac decompensation and evidence of underlying renal damage, with every indication of progressive deterioration. It was deemed advisable to attempt bilateral complete adrenalectomy in the hope of arresting or decelerating the progress of his hypertensive vascular disease.

On July 26 the left adrenal was removed. This gland measured 76 by 28 by 16 mm. and weighed 5.6 gm. The only histological abnormality noted in this gland was moderately advanced arteriosclerosis. The adrenal parenchyma otherwise appeared normal. A kidney biopsy obtained at the same time was composed of renal cortex only. Approximately half of the glomeruli were moderately to markedly hyalinized, the remainder being larger than normal but not altered in structure. Most of the renal tubules were normal in appearance, but some, included within small fibrous scars, were atrophic. The walls of the interlobular arteries were moderately thickened by hyaline tissue with corresponding narrowing of the lumens. The afferent arterioles had very narrow lumens and thick walls. The focal atrophy appeared to be on the basis of arteriolar rather than arterial involvement.

The patient's postoperative course was uneventful except for a wound abscess which drained spontaneously. At the end of two and a half weeks postoperatively the venous pressure had fallen to 55 mm. saline solution, the circulation time to eighteen seconds, and the chest roentgenogram showed a decrease in the transverse heart diameter to 145 mm. His weight had dropped to 59 kg., a loss of 2 kg. On August 18 he was discharged from the hospital for a period of convalescence, being maintained on a low salt diet only.

On October 6 the patient was again distressed by nocturnal dyspnea. At the time of his second admission on October 9 the blood pressure was 176/142 mm. Hg; there was pulsus alternans, persistent cardiomegaly with a protodiastolic gallop, and hepatomegaly but no evidence of pulmonary edema or peripheral edema. The vital capacity was 2.6 L., venous pressure 70 mm. saline solution and circulation time (decholin) twenty-five seconds. Roentgenogram of the chest showed a transverse cardiac diameter of 167 mm. with prominence of the left ventricle and congestion of the pulmonary vasculature. The blood volume determined on October 19 was 5.29 L.

Following a period of rest, administration of mercurial diuretic and sodium restriction, the patient had relief of his dyspnea but no satisfactory diuresis. On November 15 removal of the right adrenal was undertaken. Microscopically and grossly, the right adrenal gland was regarded as normal, and periadrenal arterioles showed moderate to marked hyperplasia of the media. No renal biopsy was performed at this time. Aside from a period of hypotension during the induction of anesthesia, this procedure was accomplished without incident. On the first postoperative day the patient sustained an episode of fever with a temperature to 105°F. This promptly subsided however, and by the eighth postoperative day the patient was free from dyspnea and the chest was clear on auscultation. Two weeks later the diastolic gallop was no longer audible.

The patient had been maintained on desoxycorticosterone acetate (DOCA®) and cortisone acetate postoperatively but following withdrawal of the DOCA and reduction of the cortisone dosage to 25 mg. per day a diuresis of salt and water occurred. (Fig. 1.) On December 3 the blood volume was 5.99 L. and the total body water 35.4 L. (56.7 per cent of body weight). On December 13 the vital capacity was 3.4 L. and the circulation time twenty-three seconds. The cardiac transverse diameter by x-ray was 159 mm. on December 20. An ACTH test with administration of 25 mg. intramuscularly every six hours for eight doses showed only a 20 per cent fall in circulating eosinophils (from 545 to 439 per cu. mm.). On December 21 the serum potassium concen-

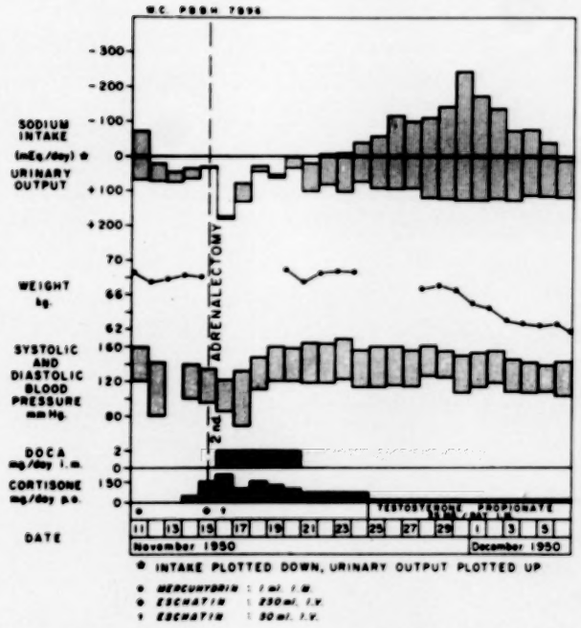

FIG. 1. Sodium diuresis following bilateral adrenalectomy.

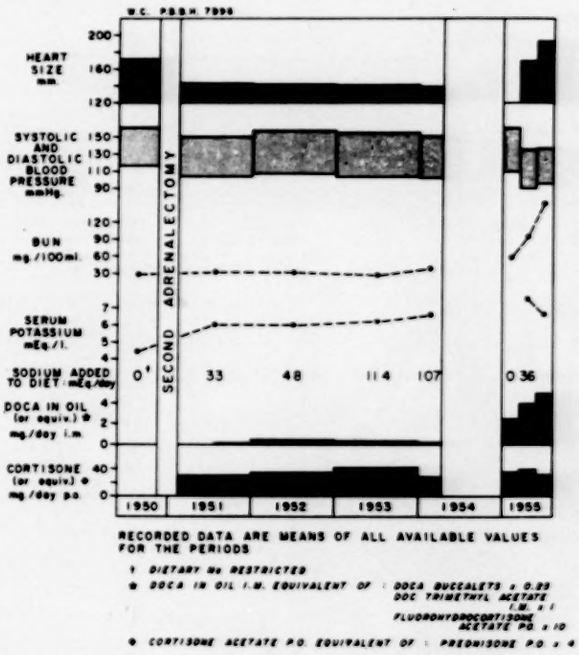

FIG. 2. Course following bilateral adrenalectomy: improved cardiac function, progressing renal damage.

tration was 4.9 mEq. per L. The patient was discharged five and a half weeks after removal of the second adrenal, receiving 0.25 mg. Digoxin daily, 25 mg. cortisone acetate daily, and 2.5 mg. DOCA intramuscularly twice a week. His salt intake was unrestricted. After a period of observation the DOCA was discontinued.

The patient survived four and a half years following removal of the second adrenal (Fig. 2) and was able during much of this time to carry on his occupation as a machinist. During this period he had twelve hospital admissions and over 100 outpatient visits to the Peter Bent Brigham Hospital. The systolic and diastolic blood pressure values were little affected by adrenalectomy when sufficient hormonal replacement was administered to render the patient effective at his work. (Fig. 2.) Cardiac function, however, was clearly improved over his preoperative status for a period of more than three years. The heart size remained smaller, there was no recurrence of pulmonary congestion, and he tolerated more effort before becoming short of breath. The digitalis therapy was continued throughout the four and a half years. In June 1951, bronchial asthma developed, and recurrences of this were responsible for three hospital admissions from that time until the end of 1952. This asthma persisted in a moderate degree during the subsequent two and a half years.

In the course of his outpatient visits the patient complained of gradually increasing periods of weakness and nausea, usually occurring on very warm days or following excessive exertion. Hyperkalemia (7.5 mEq. per L.) was documented during such an episode of weakness in August 1951, and values exceeding 6 mEq. per L. were found frequently thereafter. (Fig. 2.) Initially, both the symptoms of weakness and the elevation of serum potassium concentration could be corrected by providing a greater sodium intake, but eventually it became necessary to use DOCA to control the hyperkalemia. Increasingly large doses were necessary to maintain the patient sufficiently free of symptoms to work effectively. It is to be noted that elevation of the blood urea nitrogen level persisted following adrenalectomy (mean values during 1950 through 1953 were 29 to 32 mg. per cent) with further increases beginning in 1954 (Fig. 2) and very high values terminally.

During April 1955, while he received 0.25 mg. fluorohydrocortisone acetate by mouth daily and 50 to 75 mg. of cortisone daily, shortness of breath developed which was not relieved by bronchodilating agents. He was admitted again to the Peter Bent Brigham Hospital on April 26, 1955, at which time examination revealed evidences of congestive failure, with cyanosis, dullness and moist rales at both lung bases, displacement of the apex beat to the left anterior axillary line, a protodiastolic gallop rhythm, blood pressure of 125/90 mm. Hg with pulsus alternans, and a liver edge palpable 2 fingerbreadths below the right costal margin. There was no detectable peripheral edema. Urinalysis revealed a trace of protein but no significant sediment. The hematocrit was 31 per cent, the blood urea nitrogen was 100 mg. per cent, total protein 5.1 gm. per cent, sodium 126 mEq. per L., potassium 6.4 mEq. per L., bicarbonate 13.5 mEq. per L. and phosphorus 2.1 mM. per L. The venous pressure was 225 mm. of saline solution, circulation time

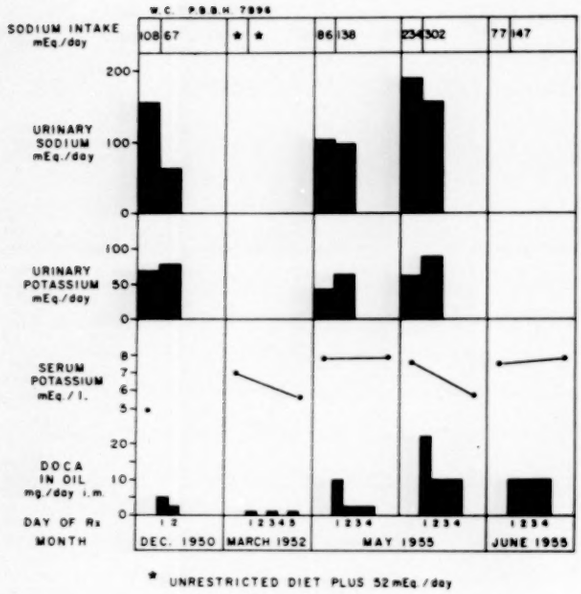

FIG. 3. Decreasing responsiveness to DOCA.

twenty-nine seconds, and the chest x-ray film showed evidence of pulmonary edema with a transverse cardiac diameter of 180 mm. The electrocardiogram revealed left bundle branch block with intervals of sinus arrest followed by nodal escape beats.

The fluorohydrocortisone was discontinued, the digitalis dose increased and fluids restricted. These measures were followed by decrease of dyspnea, a reduction in pulmonary congestion and heart size, a return of cardiac rhythm to a normal mechanism, and a weight loss of 4.5 kg. On April 30, however, there was an episode of weakness, nausea, sweating, wheezing respirations and bradycardia associated with a rise of serum potassium concentration to 10.8 mEq. per L. These symptoms subsided following an intravenous infusion of 10 per cent glucose containing 50 units of crystallin zinc insulin and 0.2 gm. of aminophyllin; 2.5 mg. of DOCA was given intramuscularly. Ten hours later the serum potassium level had fallen to 7.5 mEq. per L., and the electrocardiographic findings of moderate potassium intoxication had partially disappeared. From May 1 to 5 inclusive, daily injections of DOCA were given but there was no further drop in serum potassium concentration. (Center column, Fig. 3.) Because of progressive azotemia the dietary protein was restricted to 94 gm. per day and later reduced to 58 gm. per day. (Fig. 4.) An intravenous infusion of desoxycorticosterone on May 9 had little effect on urinary sodium excretion or serum potassium concentration. (Fig. 4.)

During the night of May 11 there was a second episode of marked weakness with malaise, sweating, irregular heart rhythm and electrocardiographic changes of potassium intoxication, all of which improved rapidly during an intravenous infusion of glucose and insulin. The serum potassium level

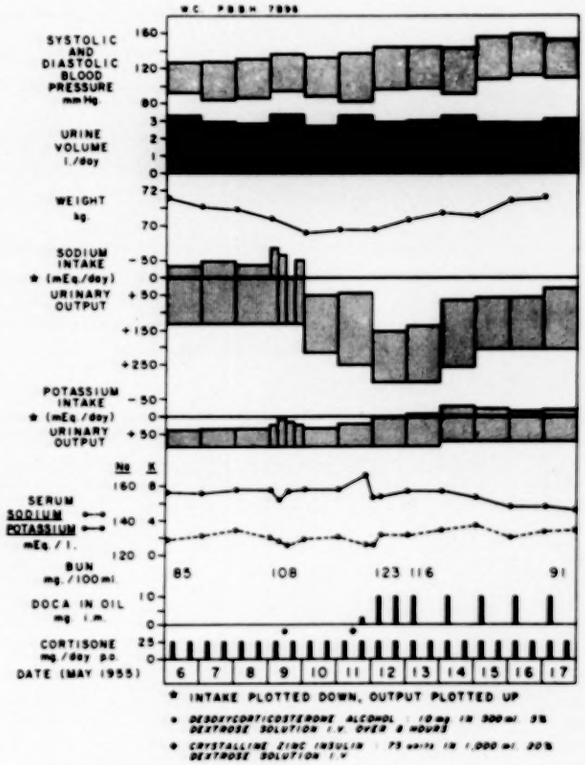

FIG. 4. Effect of withholding followed by large doses of desoxycorticosterone acetate.

obtained at the height of this episode was 9.1 mEq. per L. (Fig. 4.) Sodium intake had been increased on May 10 and 11, and it is noteworthy that the sodium balance was probably positive on the two days preceding symptomatic hyperkalemia. (Fig. 4.) Thereafter the patient received 10 mg. of DOCA daily, and there was a gradual drop of the serum potassium level to 5.4 mEq. per L. This was accompanied by urinary potassium outputs as high as 98 mEq. per day. The consistently large urine volume and the increases of weight and blood pressure following resumption of DOCA therapy are noteworthy. (Fig. 4.) The patient's total body water content (38 L. by deuterium oxide solution) and approximate extracellular fluid content (11.3 L. by twenty-minute radiosodium space) [7] were measured on May 11. Determinations of twenty-four-hour exchangeable sodium (2,500 mEq.) and potassium (3,420 mEq.) content [8] were completed on May 12.

On May 23 the patient was discharged weighing 71.5 kg. with clear evidence of pulmonary congestion. The blood urea nitrogen concentration was 80 mg. per cent, and the serum potassium level 5.8 mEq per L. The latter was maintained below 6 mEq. per L. for three weeks with fluorohydrocortisone in doses between 0.2 and 1.0 mg. daily, with supplementary cortisone or prednisone. There was a gradual gain in weight from 72 to 78 kg. with ultimate recurrence of signs of congestive heart failure. The patient was

readmitted to the hospital on June 7 and given a low sodium diet. Three injections of Mercuhydrin® were followed by a weight loss of 3 kg. However, symptoms and laboratory evidence of hyperkalemia recurred. This proved refractory to large doses of DOCA. (Fig. 3.) On June 24, 1955, the patient died with evidence of uremia, potassium intoxication and congestive heart failure.

It is of interest to note that despite the rather large maintenance dose of cortisone (50 to 75 mg. daily) which this patient received, increasing pigmentation developed following adrenalectomy so that at the time of his death his skin color was as dark as that seen in the most pronounced cases of untreated Addison's disease. We have also observed such progressive changes in patients with adrenal cortical hyperactivity who have undergone complete bilateral adrenalectomy for the relief of Cushing's syndrome. It is not known whether the increase in pigmentation represents the effect of excessive ACTH or MSH (melanocyte-stimulating hormone) secretion, which is not controlled adequately by the oral administration of cortisone and its derivatives; whether the loss of the adrenal medullary hormones is responsible; or whether unknown factors are involved. At autopsy the pituitary of this patient revealed hyperplasia of the amphophilic cells of the anterior lobe with a reduction in the eosinophilic and basophilic cells. These findings have also been reported in patients who died from Addison's disease or following bilateral adrenalectomy [*10*]. It has been suggested that the amphophilic cells are responsible for the secretion of ACTH.*

Autopsy revealed the body of a well developed, moderately obese white male with slight facial edema, acneiform eruption over the thorax, diffuse cutaneous melanotic pigmentation most marked in the body folds, and marked edema of the lower extremities extending up to the genitalia and sacrum.

Each pleural cavity contained 200 cc. of clear yellow fluid, and the peritoneal cavity contained 2,000 cc. of clear yellow fluid. The heart was markedly enlarged, weighing 575 gm. (normal weight for a male of this size is 325 to 350 gm.). All the cardiac chambers were dilated, and the left ventricular wall was moderately hypertrophied (15 mm.). The coronary arteries were moderately arteriosclerotic, and a small posterior branch of the left circumflex coronary artery was occluded by atheroma. The corresponding portion of myocardium was replaced by a fibrous scar measuring some 1.5 cm. in diameter and 0.4 cm. in thickness. Small areas of fibrosis were distributed diffusely throughout the myocardium. The lower lobes of the lungs were slightly congested. The intima of the aorta was replaced throughout most of its extent by numerous ulcerated atheromatous plaques.

* It is of interest also that the occurrence of an adenoma of the anterior lobe of the pituitary has been recently reported following bilateral total adrenalectomy for Cushing's syndrome [*14*].

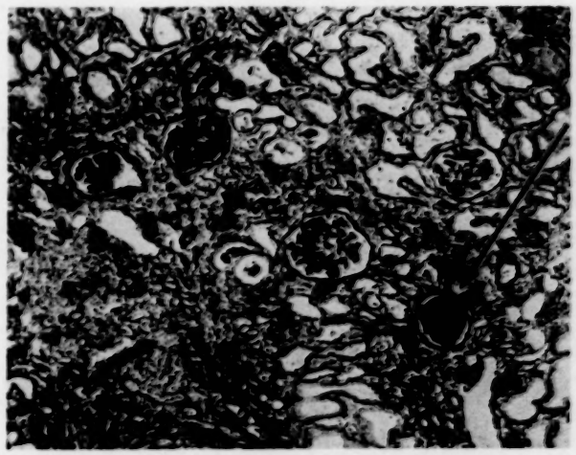

FIG. 5. Low magnification of kidney at autopsy illustrates the pattern of atrophy produced by arteriosclerosis of the smaller arteries. There is a large atrophic area occupying much of the left half of the field. The glomerular involvement is not uniform as in arteriosclerosis, which usually results predominantly in small foci of atrophy. Two essentially normal glomeruli are in the right upper quadrant; a partially hyalinized glomerulus (see arrow) with adjacent atrophic tubules is in the right lower quadrant. Periodic acid-Schiff stain with hematoxylin.

The kidneys were small, the right weighing 80 gm. and the left 100 gm. (normal weight, 150 gm. each). The kidney capsules were firmly adherent to the surface of the cortex, which was irregular due to the presence of numerous regular, slightly yellow granules which measured 1 to 2 mm. apiece, separated by flat, atrophic areas, up to 4 to 5 mm. in diameter. Upon cut section the cortex was quite narrow (2 to 3 mm.), few glomeruli could be visualized and the cut surface was mottled by numerous round, yellowish tan nodules. The corticomedullary junction was well demarcated. The medulla was of normal width and appearance. The medullary rays were slightly congested and the papillae were unremarkable. The renal arteries were patent and the walls of the interlobar branches were moderately thickened. The arcuate and interlobular arteries were not prominent. The calyces, pelves and ureters showed no abnormalities.

No residual adrenal tissue could be found. The remainder of the endocrine system appeared unaltered.

Microscopically, small foci of fibrosis which replaced the muscle fibers were diffusely spread throughout the myocardium. The posterior wall of the left ventricle was scarred by dense fibrous tissue. In the lungs the alveolar capillaries were slightly congested and small groups of alveoli contained edema fluid, macrophages laden with hemic pigment, and showed slight fibrous thickening of their septa.

The microscopic architecture of the kidneys was markedly altered due to irregular thinning of the cortex. There were alternating areas of atrophic and scarred groups of nephrons surrounded by fibrous tissue and lymphocytes, and hypertrophied and di-

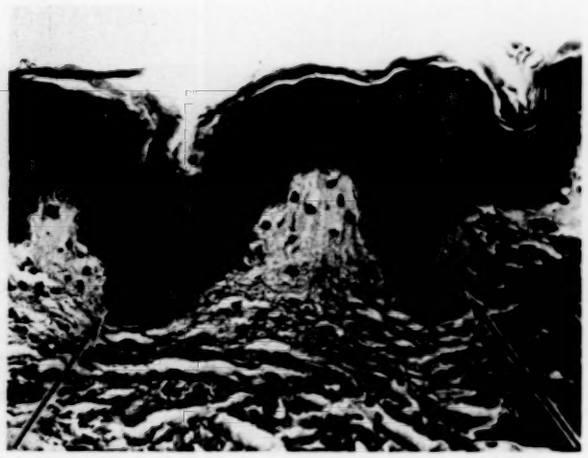

FIG. 6. Skin. There is an abnormal increase in melanin pigment in the basal layer of the epidermis. Arrows indicate the more heavily pigmented cells. Hematoxylin and eosin stain.

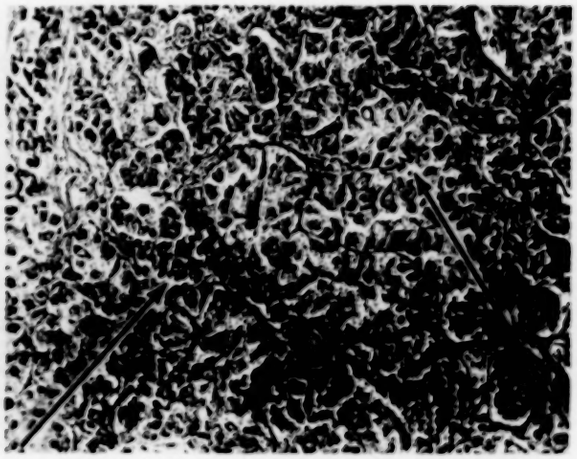

FIG. 7. Anterior lobe of the hypophysis. The predominant cell is the hypertrophic amphophil. There are multiple foci of adenomatous hyperplasia of these cells in this field, one of which is indicated by the arrow on the right. No acidophil or basophil cells are present. A small group of chromophobe cells is indicated by the arrow on the left. Periodic acid-Schiff stain; orange S.

lated nephrons, which combined to give the cortex a granular appearance. (Fig. 5.) Over half of the glomeruli were moderately to markedly hyalinized. The remainder of the glomeruli were altered to varying degrees: the capsules were slightly to moderately thickened, the capsular spaces had few adhesions and the tufts were slightly hyalinized, although the glomerular capillaries remained patent. Many of the glomeruli were much larger than normal. The arcuate and interlobular arteries showed moderate to marked sclerosis of the intima and media, with considerable thickening of their walls and reduction of their lumens. The afferent arterioles were thick, sclerotic and had narrow lumens.

Within the fibrous cortical scars were numerous atrophic tubules lined by flattened epithelial cells. The remainder of the tubules were dilated, lined by large cells with abundant eosinophilic cytoplasm and contained fluffy protein casts. The interstitial connective tissue of the cortex was sclerotic and increased in amount. Numerous lymphocytes were dispersed within it.

The process responsible for the atrophy of the kidneys was the vascular involvement in the form of arteriolosclerosis and arteriosclerosis involving the smaller arteries. The major renal arteries and their orifices were not significantly involved by arteriosclerosis.

In the spleen, the red pulp was markedly congested and contained abundant intra- and extracellular hemic pigment. The lymphoid follicles and sinusoidal cells were moderately hyperplastic. Small foci of extramedullary hematopoiesis were found in the red pulp. Examination of the liver disclosed moderate centrolobular venous and sinusoidal congestion. The skin contained increased amounts of brownish black pigment in the basal epidermal cells. (Fig. 6.)

The hypophysis had been noted to be normal in size macroscopically but the microscopic pattern of the anterior lobe was distinctly abnormal. There was a marked reduction in the basophil and eosinophil cells and the amphophil cells were markedly increased. In some portions the hyperplasia of the amphophil cells resulted in an adenomatous pattern. (Fig. 7.)

The abnormality in the microscopic pattern in the pancreas consisted of focal hyperplasia of the acinar tissue and the islands of Langerhans. The central nervous system, thyroid, marrow, breast and testis were unremarkable.

In summary, the autopsy disclosed a large heart with dilatation and hypertrophy of the ventricles, particularly the left, with small foci of myocardial fibrosis and with a small, healed left ventricular infarct. Both kidneys were small due to advanced diffuse nephrosclerosis, predominantly arterial rather than arteriolar. The aorta was lined by ulcerated atheromatous plaques, and the coronary arteries were moderately narrowed by atheromatous plaques, with occlusion of a small branch of the left anterior descending coronary. A moderate degree of chronic passive congestion of the lungs was also found.

The review of the biopsy specimens revealed that the patient had moderate to advanced arteriosclerosis involving the adrenal capsules and the kidneys, with approximately half of the glomeruli showing a degree of involvement that would be associated with reduced function. Comparison of the autopsy findings with those of the biopsy showed that there had been no apparent progression of the arteriolosclerosis. This was borne out by the finding of approximately the same number of hyalinized individual glomeruli in the autopsy and biopsy specimens. It was plain from the gross and microscopic appearance of the kidneys at

autopsy that the involvement of the small arteries, that is, the interlobular arteries, had progressed during the five-year period and had resulted in atrophy of groups of nephrons. In these arteries there was narrowing of the lumen with intimal proliferation, duplication of the elastic laminas and moderate atrophy of the media.

The major change occurring during the period between adrenalectomy and death was a progression of the arterial involvement which produced a lobular atrophy, with the consequent reduction in kidney function. This lobular atrophy resulting from arteriosclerosis of the interlobular arteries led to loss of the tubular portions of many nephrons. This differed from the change more often associated with hypertension, in which there is progression of arteriolosclerosis with atrophy of individual nephrons. (Fig. 5.)

The abnormalities noted in the skin in the form of an abnormal increase in melanotic pigment in the basal layer of the epidermis, the hyperplasia of the amphophil cells in the anterior lobe of the hypophysis with a reduction in the eosinophil and basophil cells, and the focal hyperplasia of the acinar tissue and the islands of Langerhans in the pancreas can all be regarded as sequelae of bilateral adrenalectomy. They have been observed by Dr. A. B. Russfield with whom this case was reviewed [*10*].

COMMENTS

Postoperatively this patient required gradually increasing amounts of sodium and sodium-retaining hormone to control adequately salt and water loss, hyperkalemia, azotemia and symptoms associated with these disturbances. The presence of renal disease in combination with the increasing requirement of sodium and sodium-retaining hormone may have played an important role in preventing a hoped-for reduction in arterial blood pressure. It seems probable, however, that the prolonged absence of heart failure, which had been so evident preoperatively, resulted in part from an over-all reduction in salt-retaining hormone following bilateral complete adrenalectomy. Unfortunately the technic for measuring urinary levels of aldosterone was not available during the period prior to adrenalectomy in this patient.

There was no evidence to suggest progression of myocardial impairment during the first three postoperative years, and it seems likely that the major determinant of the increasing requirement for salt and DOCA was progressive renal damage. It is not certain in what manner the progressive renal damage led to hyperkalemia, but any proposed explanation should be compatible with the absence of any gross

TABLE 1

RATIO OF EXCHANGEABLE POTASSIUM CONTENT TO OTHER INDICATORS OF BODY COMPOSITION [*8,9*]

	Patient (May 11–12, 1955)	Normal Mean
K_e/weight	49	47
$K_e/H_2O_{(D_2O)}$	89	76
$K_e/H_2O_{(D_2O)} - H_2O_{(Na_e{}^{20'})}$	126	105
K_e/weight $- H_2O_{(D_2O)}$	109	124
K_e/Na_e	1.37	1.23
K_e/creatinine	2.27	2.05

NOTE: K_e = mEq./24 hr. exchangeable body potassium content.
Na_e = mEq./24 hr. exchangeable body sodium content.
Weight = kg. body weight.
$H_2O_{(D_2O)}$ = L./3 hr. distribution volume deuterium oxide.
$H_2O_{(Na_e{}^{20'})}$ = L./20 min. distribution volume radioactive sodium (Na^{24}).
Creatinine = mg. creatinine excreted in 24 hr.

deviation from the normal of the exchangeable potassium content per unit of intercellular water [$K_e/H_2O_{(D_2O)} - H_2O_{(Na_e{}^{20'})}$] (Table 1) or per unit of excreted creatinine (Table 1) on May 11 and 12, 1955. Although these values are 120 per cent and 110 per cent of the normal mean values, respectively, they fall within the range observed in the absence of hyperkalemia in this laboratory. In interpreting the other data in Table 1, the probable slight increase of body fat content and the slight decrease of exchangeable sodium and extracellular fluid must be taken into account.

Although values for cellular potassium concentration appeared to approach normal limits, the ratio of cellular potassium concentration [approximated by $K_e/H_2O_{(D_2O)} - H_2O_{(Na_e{}^{20'})}$] to serum potassium concentration was as low as 0.6 of the mean normal value. It would seem likely, then, that the reduction of the normal gradient of potassium concentration across the cell membranes of the body was in large measure responsible for the hyperkalemia. It is not known to what extent any given organ participated in this abnormality. It is suggested that abnormal retention of metabolites resulting from impaired renal function adversely affected cellular metabolism [*11*] with a subsequent reduction of the potassium gradient across the cellular membranes. If the observations made on May 11 and

12 (Fig. 4) are accepted as representative, no shift in the potassium excretion or intake can be considered directly responsible for the patient's chronic hyperkalemia. It could be argued then that whenever hyperkalemia is associated with a normal or diminished cellular potassium content, therapeutic measures should be directed toward improving general cellular function rather than toward establishing a negative potassium balance. Any attempt to lower the serum potassium level by any method designed to deplete the extracellular fluid of this cation rapidly would be frustrated by the rapid exchange of potassium across the cell membranes from the area of high intracellular concentration. In the case reported, when hyperkalemia was corrected with salt-retaining hormone the gradient of potassium concentration across the cell membranes of the body probably was affected directly by the hormone or by some mechanism other than the degree of sodium retention.

It would appear that the patient's renal tubular capacity to respond to administration of DOCA by increased sodium reabsorption was impaired. This would render him more susceptible than otherwise to a negative sodium balance in the presence of a low sodium intake. However, Nickel and co-workers have demonstrated that in advanced nephrosclerosis and other chronic renal disorders such a trend may soon be arrested by a marked drop of sodium excretion and rise of blood urea nitrogen level, both the result of a striking further reduction in glomerular filtration rate [*12*].

The tubular potassium-secreting activity of each functioning nephron varies inversely with the glomerular filtration rate in patients with advanced nephrosclerosis and may be greatly increased when the latter is reduced. In the case reported herein, the requirement for salt-retaining hormone varied directly with the degree of renal insufficiency. One may speculate from these observations, which suggest an increased need for salt-retaining steroid, that endogenous secretion of salt-retaining steroid would be elevated in uremia resulting from a variety of renal disorders. If the secretion of aldosterone or some other highly potent sodium retainer is found to be increased in renal failure, the answers to two questions become of great interest: (1) To what extent is the hypertension so commonly observed in terminal renal failure due to this adrenal cortical secretion? (2) Is the elevated aldosterone excretion recently reported for patients with essential hypertension [*13*] correlated with—and perhaps secondary to—the degree of renal damage?

SUMMARY

The history is reported of a thirty-four year old white man who survived four and a half years following bilateral total adrenalectomy for cardiac failure due to hypertension. Marked improvement in circulatory status and a notable decrease in heart size was observed postoperatively although the degree of reduction in blood pressure level was minimal. Progressive renal insufficiency developed during the four and a half–year period, and as this occurred there was an increasing requirement for both salt and salt-retaining hormone. Chronic hyperkalemia with intermittent clinical episodes of acute potassium intoxication developed. However, the total exchangeable body potassium during this time was found to be within normal limits.

A review of the autopsy tissue revealed no reversal of the arteriolar disease noted in renal biopsies at the time of adrenalectomy. The rapid reversal of acute potassium intoxication by administration of DOCA suggests a direct effect of this hormone on the mechanism involved in sustaining the normal gradient of potassium concentrations across the cell membranes.

Acknowledgments: We are indebted to Dr. Francis Moore for total body water determinations; and to Drs. John Merrill, John Reardon and Donald Tucker for aid with total exchangeable sodium and potassium determinations, inulin space measurements and determination of glomerular filtration rate.

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Fifteen-Year Survival Following Surgery of Carcinoma of the Stomach*

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ALTHOUGH prolonged survival of patients with carcinoma of the stomach is notably rare, prognosis is more favorable if the lesion is resectable. This and other factors influencing survival will be discussed in relation to a patient who survived fifteen years following resection of a symptomatic and apparently highly invasive gastric carcinoma.

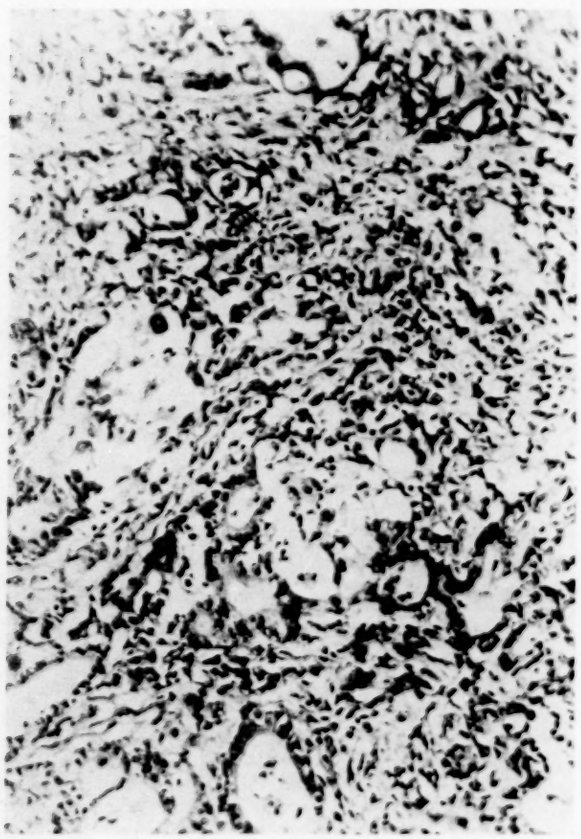

FIG. 1. Low power view of tumor.

CASE REPORT

J. S. (Bellevue Hospital Case 36150-55), a seventy-six year old white, Russian born Jewish man, was first seen by his private physician in 1939, at the age of sixty, with a complaint of persistent indigestion relieved by sodium bicarbonate. The following year gastrointestinal fluoroscopy revealed no abnormality. Symptoms increased, however, and epigastric discomfort became pronounced. Almost all ingested food was vomited and there was a weight loss of 50 pounds. He was admitted to another hospital on May 8, 1941, where gastroscopy and a repeated gastrointestinal series revealed a fungating antral lesion. At laparotomy, in spite of the fact that the growth was extensive and invaded the omentum, it was resected with a narrow margin and gastrojejunostomy was performed. No metastases were seen in the liver or lymph nodes.

Pathological examination of the specimen revealed that the entire pyloric region was occupied by an ulcerated tumor mass 8 cm. in length and 10 cm. in circumference. The distal margin of the tumor was 4 to 8 mm. from the duodenum. Histologically, there were irregular "glandular units formed by epithelial cells showing moderate degrees of pleomorphism, gigantism, hyperchromatism and heterotopia." (Figs. 1 and 2.) There was marked desmoplasia and the tumor elements extended into the serosa and omentum. The pathological diagnosis was adenocarcinoma of the stomach, grade 4.

Following surgery, the patient had no further symptoms and periodic gastrointestional x-ray films disclosed no evidence of recurrence.

On June 28, 1955, the patient was admitted to the medical wards of Bellevue Hospital because of pulmonary edema due to arteriosclerotic heart disease. This responded to the administration of oxygen, intravenous aminophylline, parenteral morphine sulfate and digitalis. Physical examination revealed no findings relevant to his former illness except the abdominal scar of the operation. There were no enlarged lymph nodes and the liver was palpable but not enlarged. Urinalysis, hemogram and liver function tests were within normal limits. Seven stool examinations were negative for blood by guaiac test; several others taken when the patient was on a diet without meat restriction were trace to 1 plus. Proctoscopy and barium enema were normal. Two upper gastrointestinal series revealed a high subtotal

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FIG. 2. High power view of glandular tumor elements.

gastrectomy with patent gastroenterostomy stoma and well visualized afferent and efferent loops. (Fig. 3.) No mucosal abnormalities were noted. On September 16, 1955 the patient was discharged, weighing his usual 101 pounds. He remained free of gastrointestinal symptoms and essentially well until July 29, 1956, when he died unexpectedly during sleep, at home. No postmortem examination was obtained.

COMMENTS

The ominous prognosis of carcinoma of the stomach is indicated by previous studies. The highest five-year survival rate that we were able to find in the literature is 12.5 per cent, reported by Shahon and associates from the University of Minnesota [*1*]. Other, perhaps more representative figures range from 5.6 to 8.8 per cent [*2–4*].

Survival for fifteen years has been notably infrequent, accounting for 0.32 and 0.47 per cent of cases in two large series [*1,3*] of 925 and 1,264 patients with gastric carcinoma. Other sources [*4–8*] mentioned one to six survivals of this length in their series. It should be remembered, however, that a normal age-corrected population undergoes attrition due to other causes. Survival rates for such a population as quoted by Berkson and associates [*9*] are 89.0 per cent at five years, 75.2 per cent at ten years and 58.3 per cent at fifteen years.

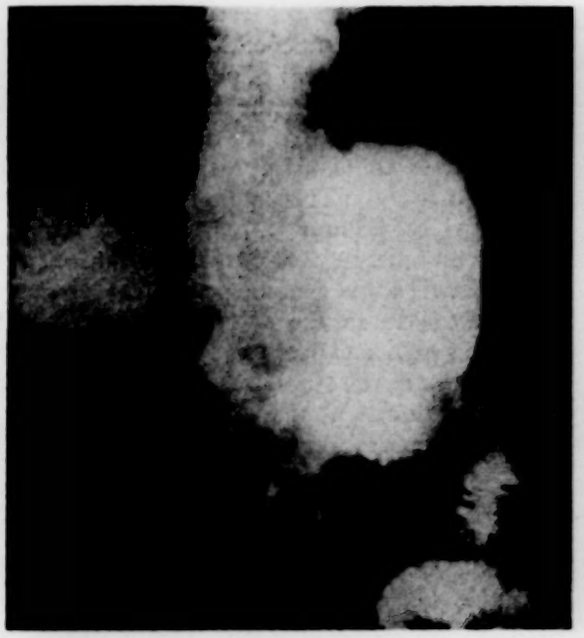

FIG. 3. Upper gastrointestinal series showing gastroenterostomy.

In spite of the possibility of longevity in this disease, the attitude of most clinicians confronted with a case such as described is one of skepticism as to the compatibility of the diagnosis with the long survival. In the case herein recorded, review of the slides dispelled all doubt. Part of the reason for this attitude may be the rarity of detailed case reports of survivals of this length. We were able to find only four such cases [*10–12*] in the English literature. The case reported herein is believed to be particularly significant in view of the anaplastic nature of the tumor and the small margin of resection.

In retrospect, there was partial justification for the initial poor prognosis. Broder's histological grading of primary tumor tissue according to anaplasia has been considered a fairly reliable rough guide to prognosis, grade 4 bearing the most ominous outlook. This degree of anaplasia and the invasion of the gastric wall and omentum, taken together with the narrow margin of resection, indicated a short survival for this patient. Other factors, however, might have provided a basis for more optimism. First, no lymph node metastases were seen at operation. Second, the fact that the lesion was resectable at all is important in spite of the narrow margin. Ransom [*3*], in his series, reported that 11.8

per cent of patients with resectable lesions survived for fifteen years. Berkson et al. [*9*] have found that 17.2 per cent of patients with resectable lesions who leave the hospital alive will survive fifteen years. Third, and perhaps most important, is the long duration of symptoms prior to diagnosis. This apparent paradox was first noted by Balfour [*13*] in 1937 and more recently by others [*2,7,14*]. In this regard, MacDonald and Kotin's monograph [*15*] presents the case for an inherent "biological predeterminism in gastric cancer" as the limiting factor of curability in resectable cases. They point out that "the duration of symptoms bears some relation to resectability, but curability increases with duration of symptoms in resectable cases." In other words, those tumors which give the longest duration of symptoms and are still resectable are the slowest growing and the most amenable to cure. An important exception would be the resectable gastric cancer which, in the guise of a peptic ulcer, might produce early symptoms.

There is other evidence that certain of these tumors undergo periods of very slow growth. Morgan and others [*16,17*] have had the opportunity to obtain repeated x-ray studies of patients who have refused operation for asymptomatic lesions suspected of being gastric carcinoma. Some of these have been followed in a "silent period" for more than three years with little change in x-ray appearance.

Other factors mentioned in the literature which seem to have less prognostic import are: location and size of the tumor, gastric acidity, age at onset, and sex of the patient.

SUMMARY

A case history of a patient with prolonged survival following resection of a symptomatic, invasive gastric carcinoma is presented. The prognosis was at first considered hopeless because of the small margin of resection and the anaplastic nature of the tumor.

While review of the literature reveals the overall outlook in this disease to be poor, several factors seem to enhance the opportunity for survival. Chief among these are resectability of the tumor, as indicated by the absence of distant metastases or extensive local spread, and low histological grade.

The concept of "biological predeterminism" is believed to be significant. Paradoxically, those tumors with the longest duration of symptoms which are still resectable have the best prognosis, apparently because of their relatively limited growth potential.

Acknowledgments: We wish to express our appreciation to Dr. Nathan Mitchell, who made slides available in this case, and to Dr. Morris Appleman, who provided us with information about the patient's course. We are indebted to Dr. Ian Gresser, who prepared the pictures of the pathological material.

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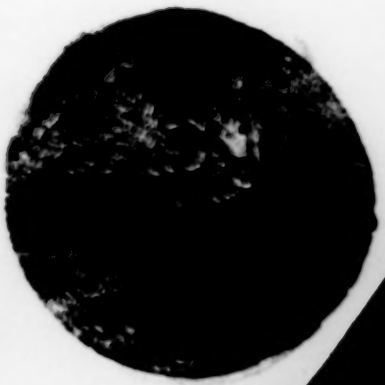

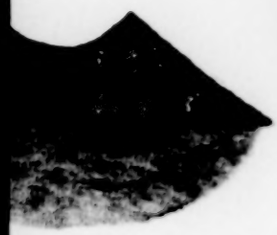

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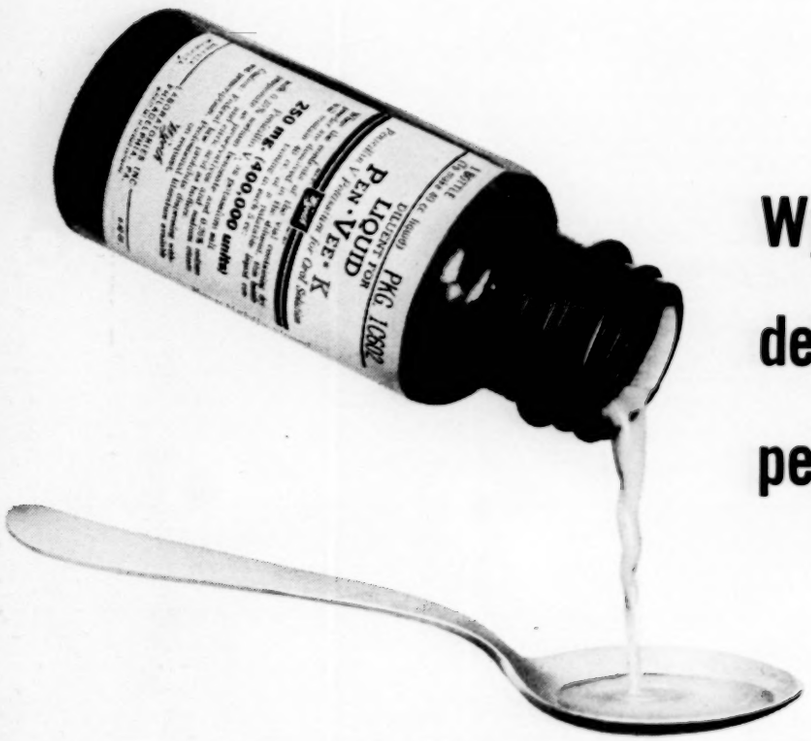

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For taste-fussy patients of all ages, Liquid PEN•VEE K gives you *two* potencies and *two* fruit flavors for flexible, patient-accepted management. It is indicated for both prophylaxis and treatment in all infections responding to oral penicillin. Ready, reliable absorption and rapid, high blood levels assure clinically effective therapeutic action. Liquid PEN•VEE K is the only liquid preparation of penicillin V potassium in two strengths and two flavors.

specific for situational stress

PHENERGAN aids in carrying your patients through difficult periods of stress. It creates a state of quiescence without depressing vital functions. Because of its many actions and uses, PHENERGAN is used extensively in obstetrics, surgery, and in wide-ranging areas of medicine.

versatile in action

Psychic sedative
Antiemetic
Antihistaminic
Analgesic and narcotic potentiator

indications:

Nausea and vomiting
Motion sickness
Surgical sedation
Obstetrical sedation
Oral surgery and dental procedures
Allergic reactions

PHENERGAN®

HYDROCHLORIDE

Promethazine Hydrochloride, Wyeth

INJECTION • TABLETS • SYRUP • SUPPOSITORIES

Comprehensive literature supplied on request

Wyeth
Philadelphia 1, Pa.

Motion sickness
Nausea and vomiting

Surgical and
obstetrical sedation

Allergic reactions

stop penicillin reactions

before they become serious

NEUTRAPEN®

(NEUTRAlizes PENicillin) (Penicillinase Injectable,* SchenLabs)

the only specific for penicillin reactions

"... if every patient with a penicillin reaction were given penicillinase [NEUTRAPEN] *within 24 to 48 hours ... I do not think we would see the severe, prolonged reactions we are seeing now."*

R. M. Becker, Antibiotics Annual 1957-58.[1]

Unlike the antihistamines, ACTH or steroids which treat effects, NEUTRAPEN, an enzyme, aborts penicillin reactions by counteracting their cause—it destroys the penicillin itself and is effective in about 97 per cent of cases.[2] Over 80 per cent of patients obtain clearing of the reaction with one injection.[3]

Obscure sources—even cases with no history of penicillin therapy respond to NEUTRAPEN when the reaction has been caused by penicillin from such sources as milk, Roquefort or bleu cheese, or penicillin containing vaccines.[3]

NEUTRAPEN—800,000 units I.M.—should be given as soon as symptoms appear. May be repeated on the third day if response is not satisfactory. In anaphylactic reactions, after routine emergency measures—800,000 units intravenously, followed immediately by 800,000 units intramuscularly.
contraindications: None. *side effects:* Occasionally transient local soreness, erythema, and edema; rarely, transitory chills and fever.
supplied: 800,000-unit single-dose vials of lyophilized penicillinase powder. Stable at room temperature in the dry state.
references: (1) Becker, R. M., in Welch, H., and Marti-Ibañez, F.: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 310. (2) Zimmerman, M. C.: Clin. Med. *5*:305, 1958. (3) Zimmerman, M. C.: J.A.M.A. *167*:1807, 1958.

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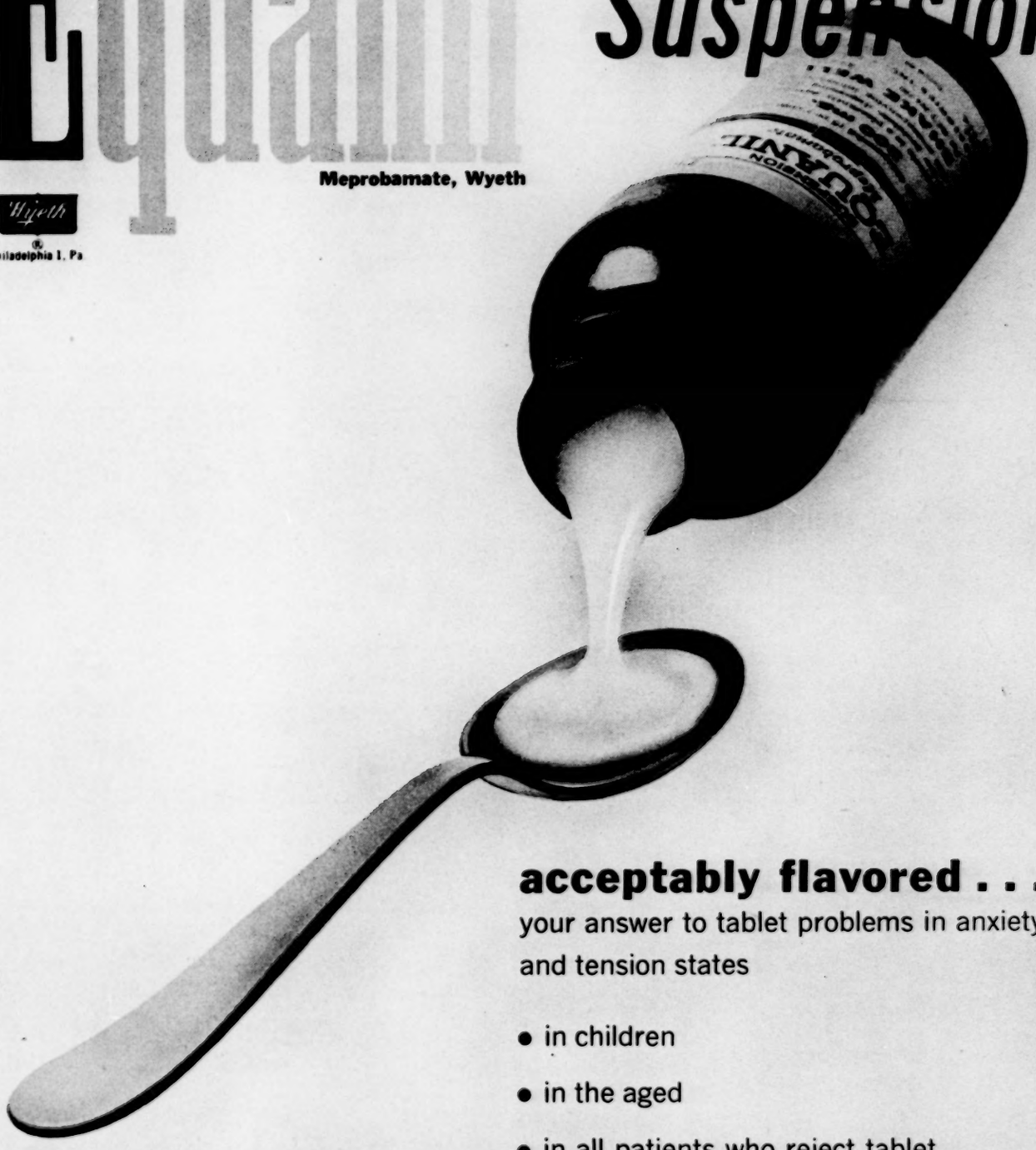
SchenLabs SCHENLABS PHARMACEUTICALS, INC • NEW YORK 1, N.Y.

now, for the first time, **liquid** meprobamate

Equanil® Suspension

Meprobamate, Wyeth

Wyeth®
Philadelphia 1, Pa



acceptably flavored . . .

your answer to tablet problems in anxiety and tension states

- in children
- in the aged
- in all patients who reject tablet medication

Conforms to Code for Advertising

SUPPLIED: Suspension, 200 mg. per 5-cc. teaspoonful, bottles of 4 fluid-ounces. Also available: Tablets, 400 mg., scored, bottles of 50; 200 mg., scored, vials of 50. WYSEALS® EQUANIL, tablets, 400 mg., vials of 50.

RELIEVES TENSION — MENTAL AND MUSCULAR

THE PIONEER IN THYROID STANDARD-IZATION

Armour thyroid tablets assure: Consistent response—unsurpassed quality—highest manufacturing standards—full potency up to 17 years of storage—dependable therapy in: frank thyroid deficiencies and when hypothyroidism is associated with chronic recurrent colds, functional menstrual disorders, sterility, habitual abortion, obesity, hypometabolism. Thyroid is recommended in long-term therapy with ACTH or corticosteroids. Supplied in $\frac{1}{4}$, $\frac{1}{2}$, 1, 2 and 5 grain strengths.

specify **ARMOUR THYROID**

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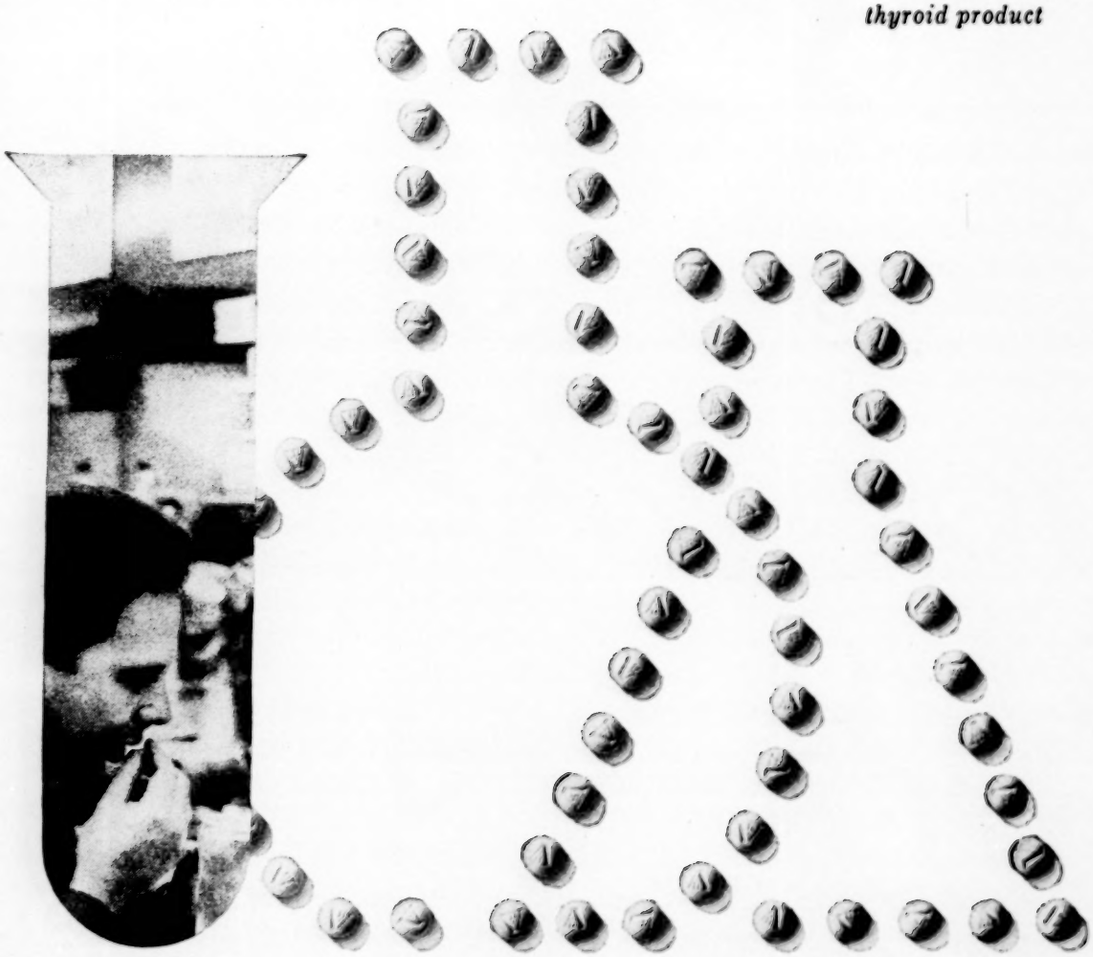
the most widely prescribed thyroid product

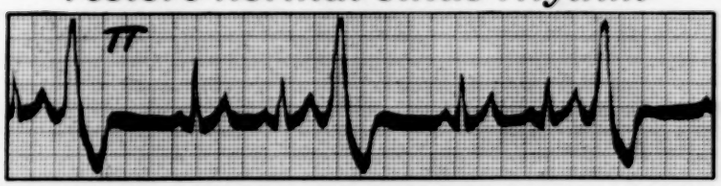


ARMOUR PHARMACEUTICAL COMPANY • KANKAKEE, ILLINOIS / *a leader in biochemical research*

Quite a surprise for this man, finding out about dandruff from his doctor. (Dandruff a disease?) But that's the paradox of the scalp-scratcher: the one man who can help him is the last man he'll ask. That's just when a word from you . . . and a prescription for SELSUN . . . will be most appreciated. Abbott

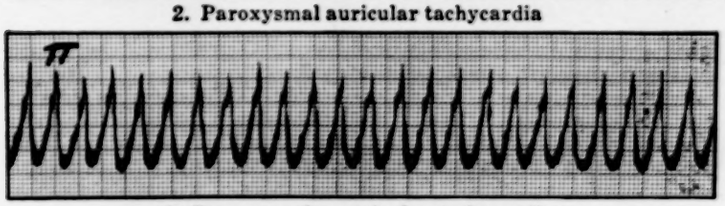
SELENIUM SULFIDE ABBOTT

restore normal sinus rhythm



1. Premature ventricular contractions

2. Paroxysmal auricular tachycardia



3. Paroxysmal ventricular tachycardia

in arrhythmias

VISTARIL*

hydroxyzine pamoate

SPECIFIC ANTIARRHYTHMIC EFFECT

Vistaril is effective in ventricular extrasystoles and paroxysmal tachycardias (both auricular and ventricular).

plus

PSYCHOTHERAPEUTIC POTENCY

proven calming action indicated for arrhythmia patients.

and

THE OUTSTANDING SAFETY

of Vistaril as compared to other antiarrhythmic drugs in general use has been noted by investigators.

THE FOLLOWING DOSAGE REGIMEN IS RECOMMENDED
(individualized by the physician for maximum effectiveness):

PARENTERAL DOSAGE: 50-100 mg. (2-4 cc.) I.M. stat., and q. 4-6 h., p.r.n.; maintain with 25 mg. b.i.d. or t.i.d. In acute emergency, 50-75 mg. (2-3 cc.) I.V. stat.; maintain with 25-50 mg. (1-2 cc.) I.V. q. 4-6 h., p.r.n.

ORAL DOSAGE: Initially, 100 mg. daily in divided doses until arrhythmia disappears. For maintenance or prophylaxis, 50-75 mg. daily in divided doses.

SUPPLY: Vistaril Capsules, 25 mg., 50 mg. and 100 mg. Vistaril Parenteral Solution, 10 cc. vials and 2 cc. Steraject® Cartridges. Each cc. contains 25 mg. (as the hydrochloride).

Pfizer *Science for the world's well-being*

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, N. Y.

References: 1. Burrell, Z. I., et al.: Am. J. Cardiol., 1:624 (May) 1958. 2. Hutcheon, D. E., et al.: J. Pharmacol. & Exper. Therap., 118:451 (Dec.) 1956.

*Trademark

Greater effectiveness: prompt and sustained relief with fewer doses. **Fewer side effects:** little or no nausea or vomiting ■ not constipating — LERITINE is spasmolytic ■ minimal unwanted CNS effects—no convulsions or similar neurologic phenomena reported in therapeutic dosage.

TABLETS: 25 mg., in bottles of 100 and 500. PARENTERAL SOLUTION: 25 mg. per cc., in 1-cc. and 2-cc. ampuls; 30-cc. vials. WARNING: May be habit-forming. NOTICE: Subject to Federal Narcotic Law.

Leritine*

(ANILERIDINE)

orally effective even for

intense pain

* LERITINE is a trademark of Merck & Co., Inc.

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DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

"Much better—thank you, doctor"

Proven in research

1. **Highest** tetracycline serum levels
2. Most consistently elevated serum levels
3. Safe, physiologic potentiation
(with a natural human metabolite)

Proven in practice

4. More rapid clinical response
5. Unexcelled toleration

COSA-TETRACYN*

GLUCOSAMINE-POTENTIATED TETRACYCLINE

CAPSULES **ORAL SUSPENSION** **PEDIATRIC DROPS**

THE *COSA*SAUR, emblem of the COSA antibiotics, symbolizes the natural origin of glucosamine—a substance widely distributed throughout the plant and animal world. Today, as in the dinosaur era, "Cosa" is basic to life.

Pfizer *Science for the world's well-being*

PFIZER LABORATORIES

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*TRADEMARK

catheters are contagious

to meet the danger/to treat the patient

Furadantin®

brand of nitrofurantoin

"We have given FURADANTIN for as long as three months to patients with indwelling catheters without deleterious effects."[1]

all things considered...

1. *The Catheter Controversy.*–"Opinions vary greatly on the danger of catheterization.... The very frequency with which the procedure is done indicates that many clinicians believe the danger to be small. At the other extreme there are experienced clinicians who regard the danger of catheterization sufficient to require stringent indications for it."[2]

2. *The Catheterization-Causation Cycle.*–"The catheter is probably the most common agent responsible for resistant urinary tract infections ... a catheter seeds the bladder with urethral bacteria."[3] "In certain parts of the country internists are claiming that most instances of chronic pyelonephritis are in patients whose infection was introduced by previous urethral catheterization."[4]

3. *The Indwelling Invitation to Infection.*–During indwelling catheterization, "the urethra is distended by a foreign body for days or weeks. The response to this is production of a sheath of mucopurulent exudate around the catheter, providing a splendid medium for growth of microorganisms. *Infection of the bladder cavity is almost inevitable* under these circumstances."[2]

4. *The Communal Catheter–A Key to Cross-Infection.*–"The most likely explanation of these findings was that there was significant cross-infection on the male surgical wards, presumed to be through the medium of instrumentation and catheterization. . . . There seems little doubt, at present, that nosocomial infections play a large role in the pathogenesis of many urinary tract infections, and that catheterization and other instrumentation are the major carriers."[5]

5. *A Culprit in Chronicity.*–"In chronic recurrent infections, catheterization and instrumentation seem to be major factors accounting for chronicity and drug failures."[6]

...there's a point to prophylaxis

"All instrumented patients, male or female, deserve prophylactic drugs to prevent iatrogenic urinary tract infections."[4] "It should be emphasized that any instrumentation should be accompanied by prophylaxis at the time of and for 24 hours subsequently to prevent unnecessary reactions, and better yet for 24 hours preceding."[7]

REFERENCES: 1. Carroll, G., et al.: J. Am. Geriat. Soc. 5:635, 1957. 2. Beeson, P. B.: Yale J. Biol. 28:81, 1955. 3. Lich, R., Jr.: J. Arkansas M. Soc. 53:271, 1956. 4. Baker, W. J.: J. Urol., Balt. 80:85, 1958. 5. Kass, E. H.: Am. J. Med. 18:764, 1955. 6. Welch, H.: The Manual of Antibiotics, New York, Medical Encyclopedia, Inc., 1955-1956, pp. 934-949. 7. Herrold, R. D.: Med. Clin. N. America 42:285, 1958.

DIURIL®

CHLOROTHIAZIDE

ANY

HYPERTENSION

RATIONALE

"It appears that there is now available in chlorothiazide a drug which is a specific antagonist to the abnormal sodium metabolism seen in the vast majority of hypertensive patients. The use of this agent [DIURIL] may stand the test of time as the most vital and specific weapon in the treatment of a relatively non-specific disease in which the only specific abnormality known is one of sodium metabolism. . . . Chlorothiazide now appears to be the drug of choice when initiating therapy in the average hypertensive patient."

Reinhardt, D. J.:
Delaware State Med. J. **30**:1, January 1958.

RESULTS

"We have presented a group of 48 patients previously treated with a variety of antihypertensive agents." "Upon the addition of chlorothiazide to their regimens, there was realized an additional blood pressure lowering effect of 23 mm. systolic and 11 mm. diastolic."

Bunn, W. H., Jr.:
Ohio State Med. J. **54**:1168, September 1958.

MINIMAL SIDE EFFECTS

"There is an extremely wide range between therapeutic and toxic dosage, and no significant side effects and no sensitivity to the drug as yet have been observed." ". . . it seems desirable to add potassium chloride 4 Gm. per day . . . in cases of hypertension. . . ."

Herrmann, G. R., Hejtmancik, M. R., Graham, R. N. and Marburger, R. C.:
Texas State J. Med. **54**:639, September 1958.

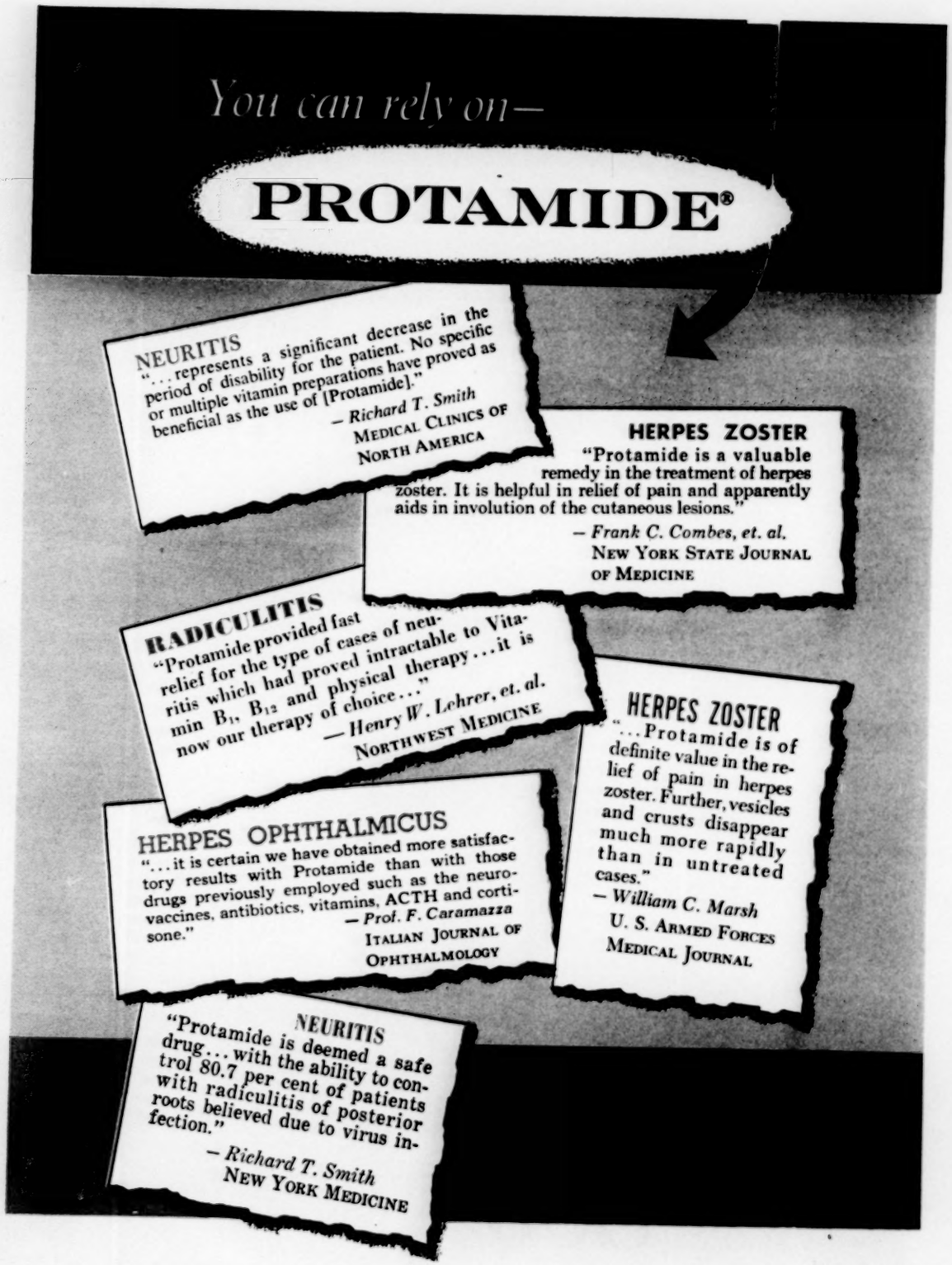
dosage: one 250 mg. tablet DIURIL b.i.d. to one 500 mg. tablet DIURIL t.i.d.

supplied: 250 mg. and 500 mg. scored tablets DIURIL (Chlorothiazide) bottles of 100 and 1000.

Your patients will say

"I slept like a log"

after taking **NEW**


You can rely on—
PROTAMIDE®
NEURITIS
"... represents a significant decrease in the period of disability for the patient. No specific or multiple vitamin preparations have proved as beneficial as the use of [Protamide]."
— Richard T. Smith
MEDICAL CLINICS OF NORTH AMERICA
HERPES ZOSTER
"Protamide is a valuable remedy in the treatment of herpes zoster. It is helpful in relief of pain and apparently aids in involution of the cutaneous lesions."
— Frank C. Combes, et. al.
NEW YORK STATE JOURNAL OF MEDICINE
RADICULITIS
"Protamide provided fast relief for the type of cases of neuritis which had proved intractable to Vitamin B1, B12 and physical therapy... it is now our therapy of choice..."
— Henry W. Lehrer, et. al.
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HERPES ZOSTER
"...Protamide is of definite value in the relief of pain in herpes zoster. Further, vesicles and crusts disappear much more rapidly than in untreated cases."
— William C. Marsh
U. S. ARMED FORCES MEDICAL JOURNAL
HERPES OPHTHALMICUS
"...it is certain we have obtained more satisfactory results with Protamide than with those drugs previously employed such as the neurovaccines, antibiotics, vitamins, ACTH and cortisone."
— Prof. F. Caramazza
ITALIAN JOURNAL OF OPHTHALMOLOGY
NEURITIS
"Protamide is deemed a safe drug... with the ability to control 80.7 per cent of patients with radiculitis of posterior roots believed due to virus infection."
— Richard T. Smith
NEW YORK MEDICINE

▲Deprol▲†

Clinically confirmed in over 2,500 documented case histories[1,2]

CONFIRMED EFFICACY

Deprol ► acts promptly to control depression *without* stimulation
► restores natural sleep
► reduces depressive rumination and crying

DOCUMENTED SAFETY

Deprol is unlike amine-oxidase inhibitors

► does not adversely affect blood pressure or sexual function
► causes no excessive elation
► produces no liver toxicity
► does not interfere with other drug therapies

Deprol is unlike central nervous stimulants

► does not cause insomnia
► produces no amphetamine-like jitteriness
► does not depress appetite
► has no depression-producing aftereffects
► can be used freely in hypertension and in unstable personalities

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

Composition: Each tablet contains 400 mg. meprobamate and 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl).

Supplied: Bottles of 50 scored tablets.

1. Alexander, L.: Chemotherapy of depression—Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. 2. Current personal communications; in the files of Wallace Laboratories.

†TRADE-MARK CO-POP

Literature and samples on request WALLACE LABORATORIES, *New Brunswick, N. J.*

more than tetracycline alone

MYSTECLIN-V CONTAINS TETRACYCLINE PHOSPHATE COMPLEX FOR A DIRECT ATTACK ON THE PRIMARY INFECTION

Mysteclin-V strikes directly at all tetracycline sensitive organisms—most pathogenic bacteria, certain rickettsias, certain large viruses, Endamoeba histolytica. It provides all established benefits of tetracycline in the new, more effective phosphate complex form.[1] Patient response is rapid because initial high peak blood serum levels may be maintained with ease at the antibacterial attack level until the infection is conquered.

BOTH ARE OFTEN NEEDED WHEN

MYSTECL

Squibb Tetracycline Phosphate Complex (Sumycin) and Nystatin (Mycostatin)

MYSTECLIN-V CONTAINS MYCOSTATIN FOR A SPECIFIC DEFENSE AGAINST SECONDARY MONILIAL SUPERINFECTION

Mysteclin-V protects patients against antibiotic induced intestinal moniliasis and its complications, including vaginal and anogenital moniliasis, even potentially fatal systemic moniliasis. This action is provided by Mycostatin, the antifungal antibiotic, with specific action against Candida (Monilia) albicans.[2]

BACTERIAL INFECTION OCCURS IN-V

Capsules (250 mg./250,000 u), bottles of 16 and 100.
Half-strength Capsules (125 mg./125,000 u), bottles of 16 and 100.
Suspension (125 mg./125,000 u per 5 cc.), 2 oz. bottles.
Pediatric Drops (100 mg./100,000 u per cc.), 10 cc. dropper bottles.

References: 1. Cronk, G. A.; Naumann, D. E., and Casson, K.: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia Inc. 1958, p. 397 • 2. Newcomer, V. D.; Wright, E. T., and Sternberg, T. H.: Antibiotics Annual 1954-1955, New York, Medical Encyclopedia Inc., 1955, p. 686.

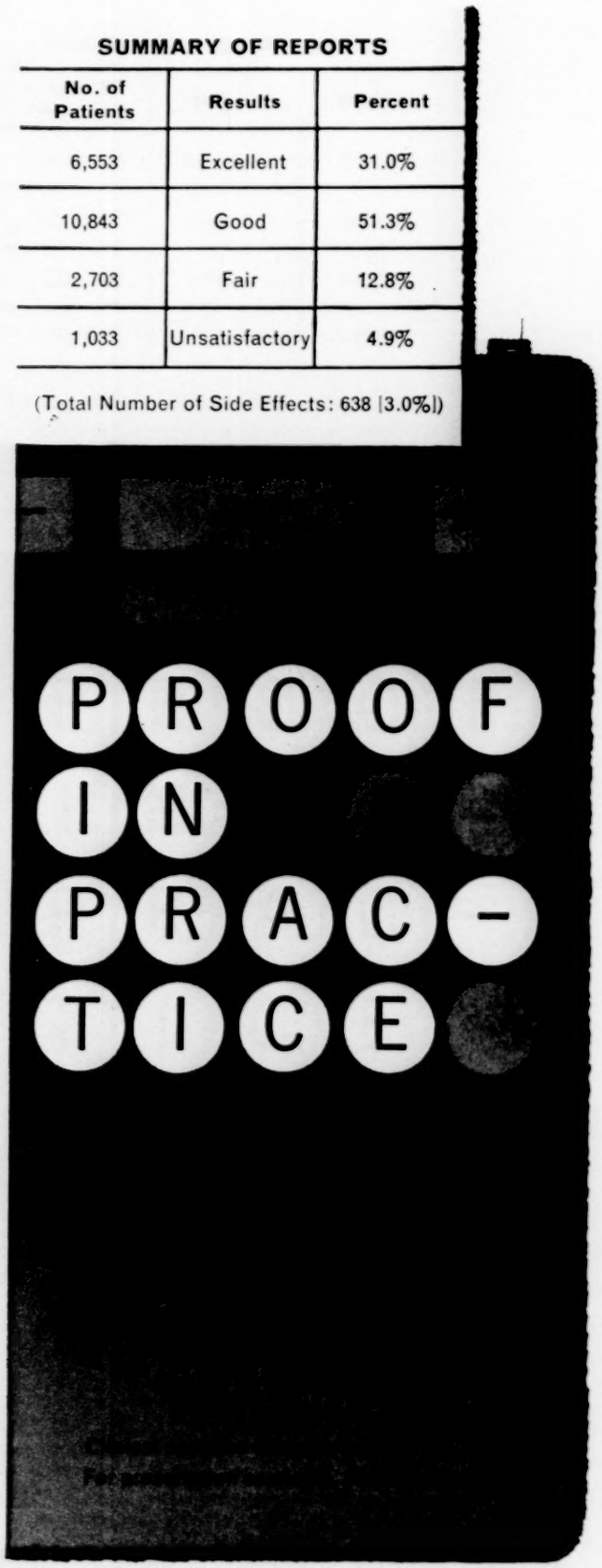
SQUIBB

Squibb Quality—the Priceless Ingredient

SUMMARY OF REPORTS

No. of Patients	Results	Percent
6,553	Excellent	31.0%
10,843	Good	51.3%
2,703	Fair	12.8%
1,033	Unsatisfactory	4.9%

(Total Number of Side Effects: 638 [3.0%])



A NEW DIMENSION IN RESEARCH

This data deals with the results obtained by 1,988 physicians, treating 21,128 hypertensive patients with Unitensen. The "Proof In Practice" study validates, *in day-to-day private practice*, the findings of clinical trials conducted in hospitals and institutions. It proves that Unitensen affords safe, dependable office management for the majority of hypertensive patients. Unitensen lowers blood pressure . . . improves cerebral and renal blood flow... exerts no adverse effects on circulation . . . and, is virtually free of side effects.

Neisler

Irwin, Neisler & Co.
Decatur, Illinois

AN ING

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DIUPRES*

DIURIL® WITH RESERPINE

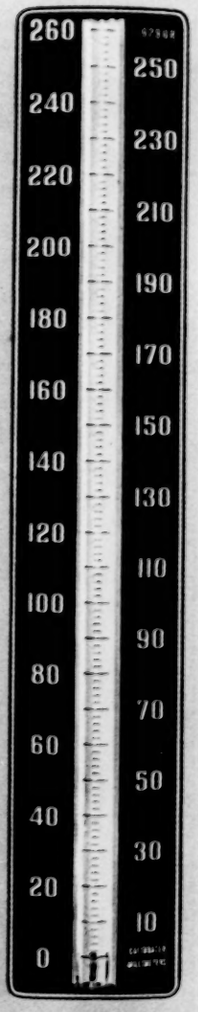
more hypertensives can be better controlled
with **DIUPRES** than with any other agent
... with greater simplicity and convenience

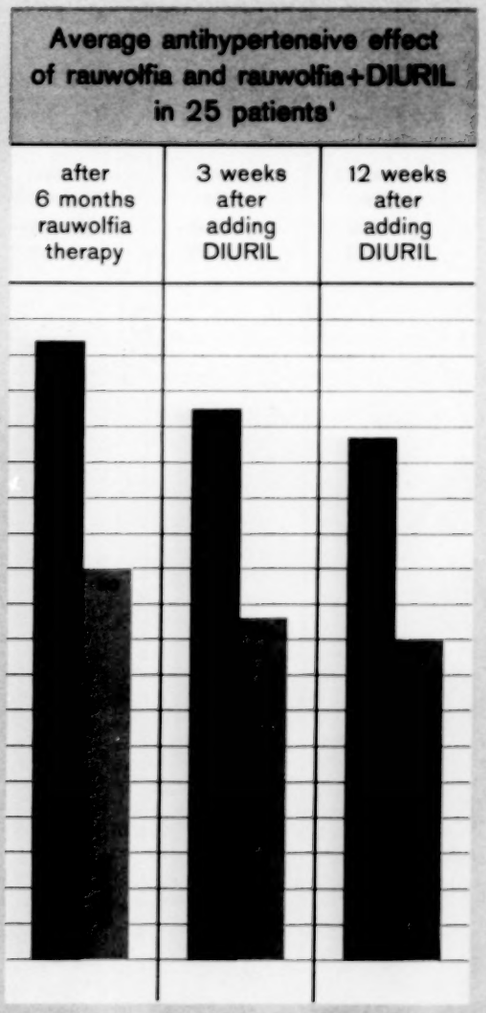
a logical alliance of two antihypertensives
you know and trust provides

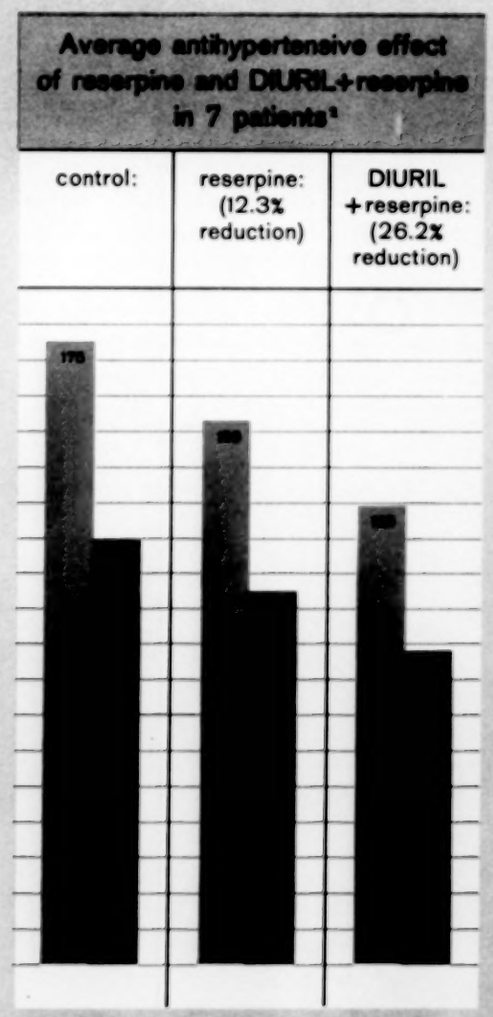
increased effectiveness, decreased side effects

potentiated effect

DIUPRES produces an effect greater than either DIURIL or reserpine alone. It is effective in many patients who respond inadequately or not at all to either DIURIL or reserpine.







DIUPRES

DIURIL® WITH RESERPINE

effective therapy for most patients

DIUPRES by itself usually provides effective therapy for a majority of patients with mild or moderate hypertension, and even for many patients with severe hypertension. Many patients now treated with other agents which frequently cause distressing side effects can be adequately managed with well tolerated DIUPRES.

provides basic therapy

Should other drugs need to be added to DIUPRES, they can be given in much lower than usual dosage so that their side effects are often strikingly reduced.

rapid onset of effect

The antihypertensive action of DIUPRES is rapidly evident. (Considerable time may elapse before the antihypertensive effect of reserpine alone is observed.)

fewer and less severe side effects

DIUPRES may be expected to cause fewer and less severe side effects than are encountered with other antihypertensive therapy. (Since DIURIL and reserpine potentiate each other, the required dosage of each is usually less when given together as DIUPRES than when given alone. Such reduction in dosage makes side effects less likely to occur.)

often obviates weight gain

DIUPRES minimizes the problem of weight gain seen with reserpine (reserpine alone has been reported to produce weight gain in 50 per cent of patients).[1,4]

virtually eliminates fluid retention

DIUPRES is not likely to cause either clinical or subclinical retention of sodium and water. (Hypotensive drugs, particularly rauwolfia[5] and hydralazine,[6] may cause fluid retention. Even when such retention is subclinical, their antihypertensive effectiveness is diminished.[6])

diet more palatable

With DIUPRES, there is less need for rigid restriction of dietary salt, which patients find so burdensome.

> *"It may well be that the drug* [DIURIL] *produces the benefits of a markedly restricted low sodium diet but without its hardships."*[3]

subjective and objective improvement

DIUPRES allays anxiety and tension, thus reducing the emotional component of hypertension. Organic changes of hypertension may be arrested and reversed. Headache, dizziness, palpitations and tachycardia are usually promptly relieved by DIUPRES. When the *anginal syndrome* accompanies hypertension, the administration of DIUPRES may also cause diminution or even disappearance of this syndrome concurrent with control of the hypertension.

convenient, controlled dosage

Instead of two separate prescriptions, you write one prescription . . . the patient takes one tablet, rather than two different tablets . . . and the dosage schedule is easier for the patient to remember and follow.

> *"patients have fewer lapses and make fewer mistakes in dosage, the simpler the regimen can be made. Therefore I do not hesitate to use more than one medicament combined in one tablet, provided this gives approximately the correct dosage of each."*[8]

economical

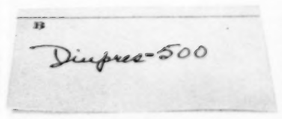
DIUPRES will cost the patient less than if he were given two separate prescriptions for its components.

Indications:
DIUPRES is indicated in hypertension of all degrees of severity. It can be used in the following ways:

- as total therapy
- as primary therapy, adding other drugs if necessary
- as replacement or adjunctive therapy in patients now treated with other agents

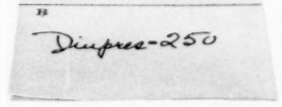
Precautions:
The precautions normally observed with DIURIL or reserpine apply to DIUPRES. Additional information on DIUPRES is available to physicians on request.

Recommended dosage range:
DIUPRES-500—one tablet one to three times a day.
DIUPRES-250—one tablet one to four times a day.
If necessary, other agents may be added.
If the patient is receiving ganglion blocking agents or hydralazine, their dosage should be cut by 50 per cent when DIUPRES is added.



DIUPRES-500

500 mg. DIURIL (chlorothiazide), 0.125 mg. reserpine.
Bottles of 100, 1000.



DIUPRES-250

250 mg. DIURIL (chlorothiazide), 0.125 mg. reserpine.
Bottles of 100, 1000.

the first "wide range" antihypertensive

DIUPRES

DIURIL® WITH RESERPINE

1. Rochelle, J. B., III, Bullock, A. C., and Ford, R. V.: Potentiation of antihypertensive therapy by use of chlorothiazide, *J.A.M.A.* 168:410, Sept. 27, 1958. 2. Freis, E. D., Wanko, A., Wilson, I. M., and Parrish, A. E.: Treatment of essential hypertension with chlorothiazide (Diuril), *J.A.M.A.* 166:137, Jan. 11, 1958. 3. Freis, E. D.: Treatment of hypertension. (Presented at the Annual Meeting of Southern Medical Association, Nov. 13, 1957.) 4. Moyer, J. H., Dennis, E., and Ford, R.: Drug therapy (Rauwolfia) of hypertension, *A.M.A. Arch. Int. Med.* 96:530, Oct. 1955. 5. Perera, G. A.: Edema and congestive failure related to administration of rauwolfia serpentina, *J.A.M.A.* 159:439, Oct. 1, 1955. 6. Wilkins, R. W.: Precautions in use of antihypertensive drugs, including chlorothiazide, *J.A.M.A.* 167:801, June 14, 1958.

MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

*DIUPRES and DIURIL (chlorothiazide) are trademarks of Merck & Co., Inc.

NEW THERAPY...TO DEFEAT THE MIGRAINE PARADOX*

'MIGRAL'®
TRADEMARK

• relieves headache
• dispels visual disturbances
and • overcomes nausea and vomiting

*The paradox of migraine—increased nausea due to ergotamine administration—may now be successfully combated with 'Migral'. The recognized benefits of ergotamine and caffeine in 'Migral' are favorably enhanced by the addition of cyclizine hydrochloride, a specific to overcome nausea.

Dosage: 2 to 3 tablets at first warning of an attack, then 1 or 2 tablets every half hour; not more than 6 tablets should be taken for any single attack.

Supplied: 'Migral' tablets, containing ergotamine tartrate 1 mg., 'Marezine'® brand Cyclizine Hydrochloride 25 mg., and caffeine 50 mg.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

- postoperatively
- in pregnancy when vomiting is persistent
- following neurosurgical diagnostic procedures
- in infections, intra-abdominal disease, and carcinomatosis
- after nitrogen mustard therapy

for nausea and vomiting

VESPRIN

Squibb Triflupromazine

- provides prompt, potent, and long-lasting control
- capable of depressing the gag reflex
- effective in cases refractory to other potent antiemetic agents
- may be given intravenously, intramuscularly and orally
- no pain or irritation on injection

ANTIEMETIC DOSAGE:
Intravenous: 8 mg. average single dose
Dosage range 2-10 mg.
Intramuscular: 15 mg. average single dose
Dosage range 5-15 mg.
If subsequent parenteral dose is needed, one-half the original dose will usually suffice
Oral: 10-20 mg. initially; then 10 mg. t.i.d.

SUPPLY:
Parenteral solution — 1 cc. ampuls (20 mg./cc.), 1 cc. multiple dose vials (20 mg./cc.)
Oral tablets — 10 mg., 25 mg., 50 mg., in bottles of 50 and 500

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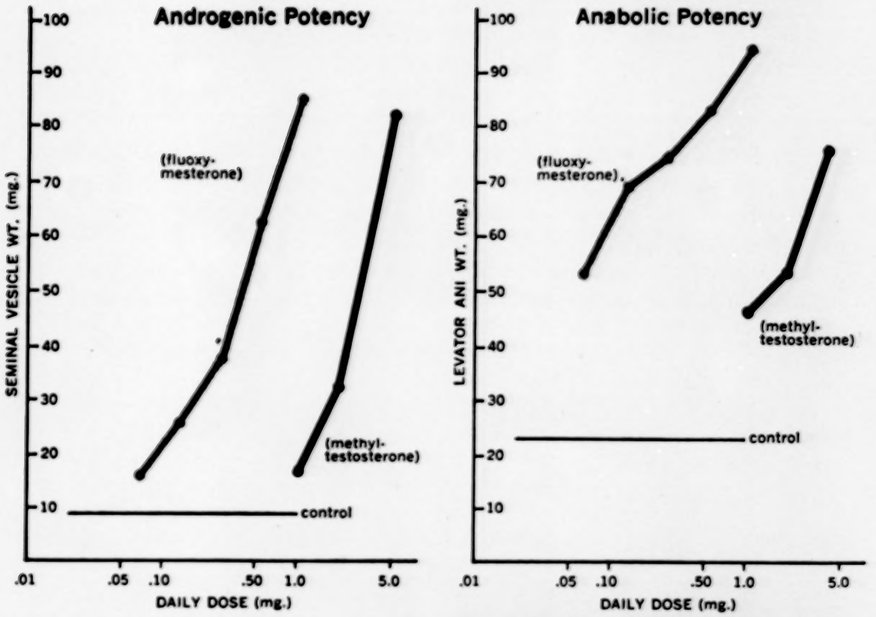
the ULTIMATE

In a comparison of androgenic and anabolic activity, clinical studies show that at least five times as many milligrams of methyltestosterone are needed to provide the same effect as fluoxymesterone.*

*Charts adapted from Lyster, S. C., Lund, G. H., and Stafford, R. O.: Endocrinology 58:781 (June) 1956.



in androgen therapy Ultandren™

(fluoxymesterone CIBA)

an oral androgen with at least five times the potency of methyltestosterone tablets, and even greater clinical potential than intramuscular testosterone preparations

Ultandren permits easily controlled administration of androgen therapy—without painful injections, local reactions or skipped doses. Weight for weight, it has an even greater therapeutic potency than parenterally administered testosterone derivatives, and at least five times the androgenic and anabolic activity of methyltestosterone tablets. Moreover, Ultandren does not increase undesirable effects such as virilism in females, and in therapeutic doses it induces little or no sodium and water retention. Jaundice, occasionally encountered with usual androgen therapy, has not been reported.

SUPPLIED: *Tablets,* 2 mg. (light-green, scored) and 5 mg. (violet, scored); bottles of 40.

C I B A
SUMMIT, N. J.

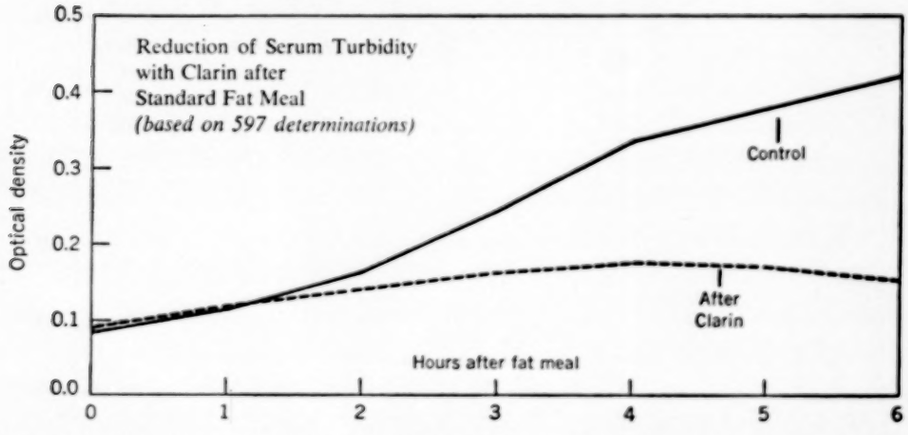
2/7639MA

in the management of atherosclerosis

Clarin*

(sublingual heparin potassium, Leeming)

clears lipemic serum



Each time your patients eat a substantial fat-containing meal, lipemia results. Small amounts of injected heparin will help control this increased fat content in the blood,[1,2] but widespread adoption of this method has been hampered by its inconvenience, pain, cost and the necessity for periodic checks on blood clotting time.

Now, long-term preventive heparin therapy is practical for the first time with the introduction of CLARIN—which is heparin in *sublingual* form. Each CLARIN tablet contains 1500 I.U. of heparin potassium—a sufficient amount to clear lipemic serum without affecting coagulation mechanisms.[3,4]

With one mint-flavored CLARIN tablet under the tongue after each meal, lipemia is regularly controlled, removing a constant source of danger to the atherosclerotic patient. He may eat safely, with less fear of dangerous results, without hard-to-follow diets.

The varied implications of CLARIN in beneficially affecting fat metabolism are obviously far-reaching. The relationship between heparin, lipid metabolism and atherosclerosis may well be analogous to that between insulin, carbohydrate metabolism and diabetes mellitus.[5]

Use CLARIN to protect your atherosclerotic patients—the postcoronaries and those with early signs of coronary artery disease.

Indication: For the management of hyperlipemia associated with atherosclerosis.

Dosage: After each meal, hold one tablet under the tongue until dissolved.

Supplied: In bottles of 50 pink, sublingual tablets, each containing 1500 I.U. heparin potassium.

1. Council on Drugs, J.A.M.A. *166*:52 (Jan. 4) 1958. 2. Hahn, P. F.: Science *98*:19 (July 2) 1943. 3. Fuller, H. L.: Angiology *9*:311 (Oct.) 1958. 4. Rubio, F. A., Jr.: Personal communication. 5. Engelberg, H., *et al.*: Circulation *13*:489 (April) 1956.

*Trade Mark. Patent applied for.

Thos. Leeming & Co. Inc.

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The hyper-anxious hypertensive needs greater central control **Serpasil®**

(reserpine CIBA)

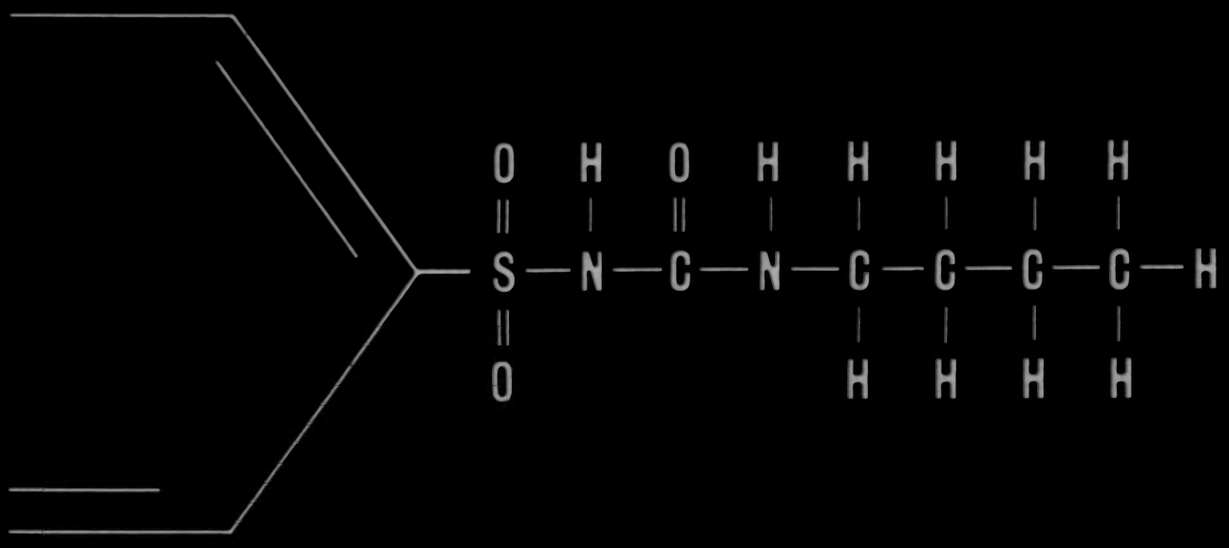
Hypertensive patients whose emotional disturbances cannot be controlled by agents which have no autonomic action need Serpasil: it acts on the central autonomic mechanisms which control reactions to stress. Thus, Serpasil not only lowers blood pressure, but eases the anxiety and agitation that contribute to your patient's hypertension.

DOSAGE: In the average patient not receiving other antihypertensive agents, the average initial dosage is two 0.25-mg. tablets daily, with a range of 0.1 to 1 mg. Later the Serpasil dosage should be reduced to 0.25 mg. or less daily for maintenance. SUPPLIED: *Tablets*, 0.1 mg., 0.25 mg., 1 mg., 2 mg. and 4 mg.

2/2430 MD

An exclusive

methyl "governor" prevents hypoglycemia... makes Orinase* a true euglycemic agent



The significant difference between Orinase and all other anti-diabetes agents is that there is virtually no danger of hypoglycemic reactions as a result of Orinase therapy, regardless of dosage.

A logical explanation is that Orinase's exclusive methyl group in the para position serves as a "governor" to prevent hypoglycemia by facilitating the rapid inactivation of the molecule in the body. There is no *cumulative* effect.

The result is that, in patients in whom maintenance dosage has been established, Orinase lowers the blood sugar *to* normal levels, but almost never *beyond* that point. In other words, Orinase is a true *eu*glycemic agent, in contradistinction to the others, which actually *are* hypoglycemic agents.

This unique margin of safety is especially important in the patient requiring insulin, because Orinase, superimposed on his insulin dosage, constitutes no added danger of hypoglycemia. This makes it feasible for you to smooth out the "peaks and valleys" of erratic blood sugar levels... to "stabilize" a surprising percentage of labile diabetics.

*TRADEMARK, REG. U. S. PAT. OFF.—TOLBUTAMIDE, UPJOHN

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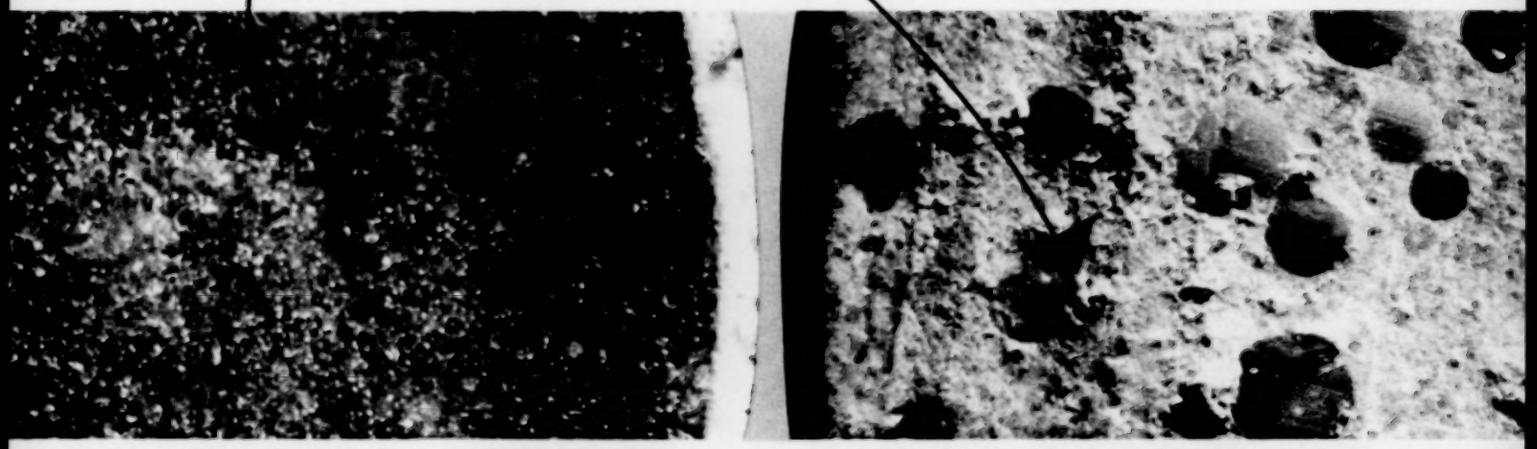

Microphotograph of a **HOMAGENET**

Microphotograph of usual vitamin product

The photographs above point out the difference between Homagenets and many vitamin products. By means of the homogenization process, both oil and water soluble vitamins are presented in microscopic particles. Due to the fine particle size, there is much greater surface exposure, which permits quicker absorption and better utilization. This has been clinically proved by Lewis and others.[1]

1. Lewis, J.M., et al.: J. Pediatrics, *31*:496

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*U.S. Pat. 2676136. Other Pat. Pending

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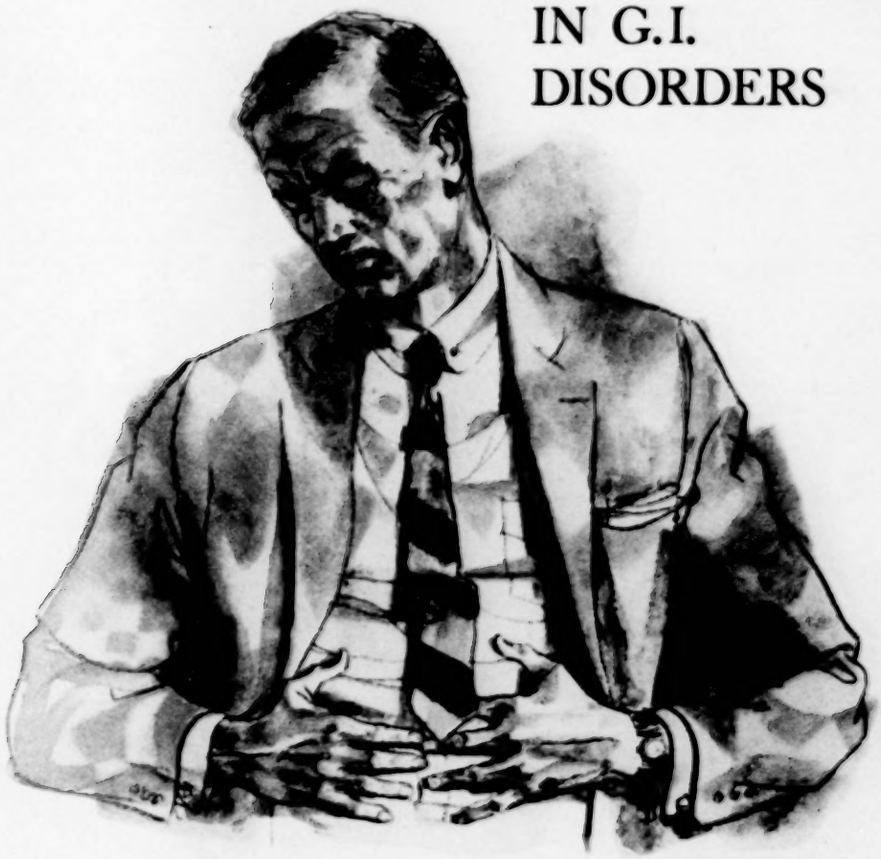
CONTROLS NERVOUS TENSION IN G.I. DISORDERS



MOST FUNCTIONAL G.I. DISORDERS "can be considered a manifestation of a general psychoneurotic disturbance." (Rossien, A. X.: J. Am. Geriatrics Soc. 5:430, April 1957.)

TREATMENT WITH MILTOWN

- improved control in 15 of 19 cases of common functional G.I. disturbances[1]
- helped the majority of 23 cases of psychosomatic stomach distress[2]
- controlled emotional components of spastic colitis,[3] chronic ulcerative colitis,[4] and psychophysiologic dyspepsia[5]

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meprobamate (Wallace)

Miltown causes no adverse effects on gastric secretions, emptying time or motility.[6]

Available in 400 mg. scored and 200 mg. sugar-coated tablets. Also available as MEPROSPAN® (200 mg. meprobamate *continuous release* capsules).

1. Phillips, R. E.: Am. Pract. & Digest Treat. 7:1573, Oct. 1956. 2. Selling, L. S.: J.A.M.A. 157:1594, April 30, 1955. 3. Altschul, A. and Billow, B.: New York J. Med. 57:2361, July 15, 1957. 4. Ross, S. T.: Postgrad. Med. 23:24, Jan. 1958. 5. Tacket, H. S.: Am. Pract. & Digest Treat. 8:597, April 1957. 6. Bodi, T., Wirts, C. W., Jr. and Menduke, H.: Am. J. Gastroenterol. 29:643, June 1958.

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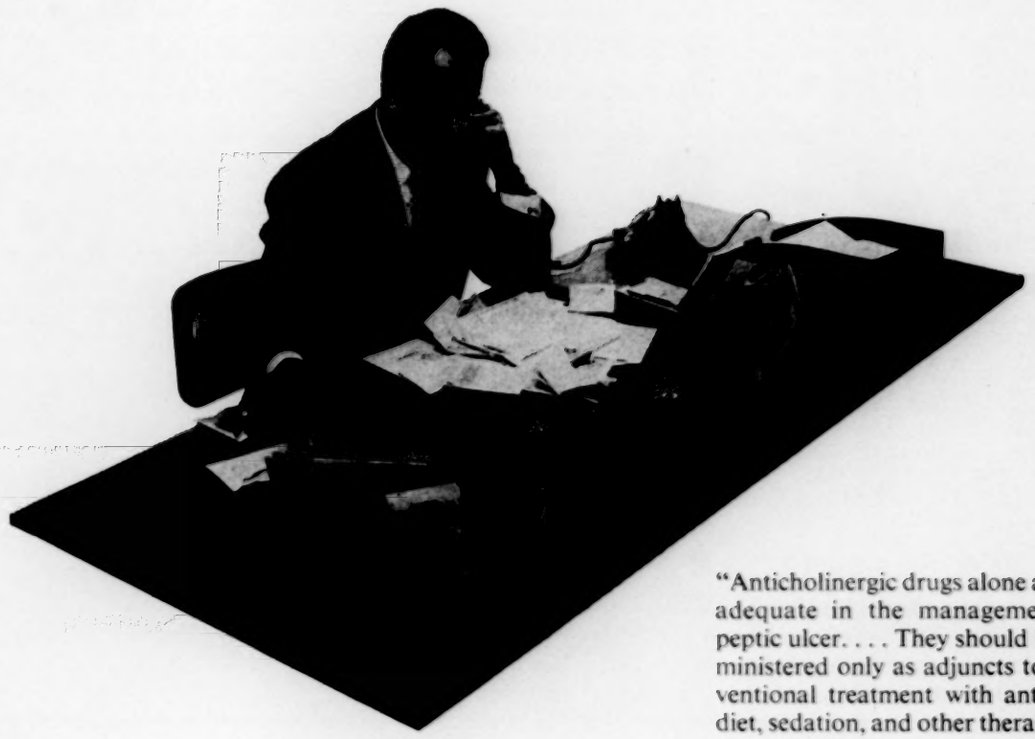

"Anticholinergic drugs alone are inadequate in the management of peptic ulcer. . . . They should be administered only as adjuncts to conventional treatment with antacids, diet, sedation, and other therapeutic measures."[1]

1. Kirsner, J.B., et al.: M. Clin. North America *41*:499 (March) 1957.

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ALUDROX SA is not only an effective anticholinergic, but also an antacid, sedative, demulcent, anticonstipant, and pepsin-inhibitor. Thus, one convenient preparation satisfies six requirements of total peptic-ulcer therapy.

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"Mommy had such an awful ache she had to go to bed"

"So I had to make dinner for Daddy. I even got the pills our doctor ordered"

"Then the pain went away real fast — almost before I finished the dishes"

"Now Mommy is fine... and she said I could help cook... but Daddy said just the tea"

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ACTS FASTER – usually within 5-15 minutes
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February, 1959

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(Waldman, S. and Pelner, L.: Management of anxiety associated with heart disease. Am. Pract. & Digest Treat. 8:1075, July 1957.)

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Toll, N.: Deaner: An Adjunct for Treatment of Schizoid and Schizophrenic Patients, Am. J. Psychiat. *115*:336 (Oct.) 1958.

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